Dissertation on

A CLINICAL STUDY OF VITAMIN D SUPPLEMENTATION IN DIABETIC RETINOPATHY PATIENTS WITH TYPE 2 DIABETES MELLITUS

Submitted in partial fulfillment of requirements of

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MADRAS MEDICAL COLLEGE
CHENNAI- 600 003

THE TAMILNADU
DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this dissertation titled "**A CLINICAL STUDY OF VITAMIN D SUPPLEMENTATION IN DIABETIC RETINOPATHY PATIENTS WITH TYPE 2 DIABETES MELLITUS**" is bonafide record of the research work done by **DR. JAIN LUBHANI SUDARSHAN,** Post graduate in the Regional Institute of Ophthalmology & Government Ophthalmic Hospital, Madras Medical College and Government General Hospital, Chennai-03, in partial fulfillment of the regulations laid down by the Tamil Nadu Dr. M.G.R Medical University for the award of M.S. Ophthalmology Branch III, under my guidance and supervision during the academic year 2012 – 2015.

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I wish to express my sincere thanks to all the professors, assistant professors and all my colleagues who had helped me in bringing out this study.

Finally, I am indebted to all the patients for their sincere co-operation for the completion of this study.

INSTITUTIONAL ETHICS COMMITTEE
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To
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Dear Dr. Lubhani Jain,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"A clinical study of Vitamin D supplementation in Diabetic Retinopathy patients with type 2 Diabetes Mellitus"** No.43062014

The following members of Ethics Committee were present in the meeting held on 03.06.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. C. Rajendran, M.D.	-- Chairperson
2.	Dr. R. Vimala, M.D., Dean, MMC, Ch-3.	-- Deputy Chair Person
3.	Prof. Kalaiselvi, MD., Vice-Principal, MMC, Ch-3	-- Member
4.	Prof. Nandhini, M.D. Inst. of Pharmacology, MMC, Ch-3.	-- Member
5.	Dr. G. Muralidharan, Director Incharge , Inst. of Surgery	-- Member
6.	Prof. Md Ali, MD., DM., Prof & HOD of MGE, MMC, Ch-3.	-- Member
7.	Prof. Ramadevi, Director i/c, Biochemistry, MMC,Ch-3.	-- Member
8.	Prof. Saraswathy, MD., Director, Pathology, MMC, Ch-3.	-- Member
9.	Prof. Tito, Director, i/c. Inst. of Internal Medicine, MMC	-- Member
10.	Thiru. Rameshkumar, Administrative Officer	-- Lay Person
11.	Thiru. S. Govindasamy, BABL, High Court, Chennai-1.	-- Lawyer
12.	Tmt. Arnold Saulina, MA MSW	-- Social Scientist

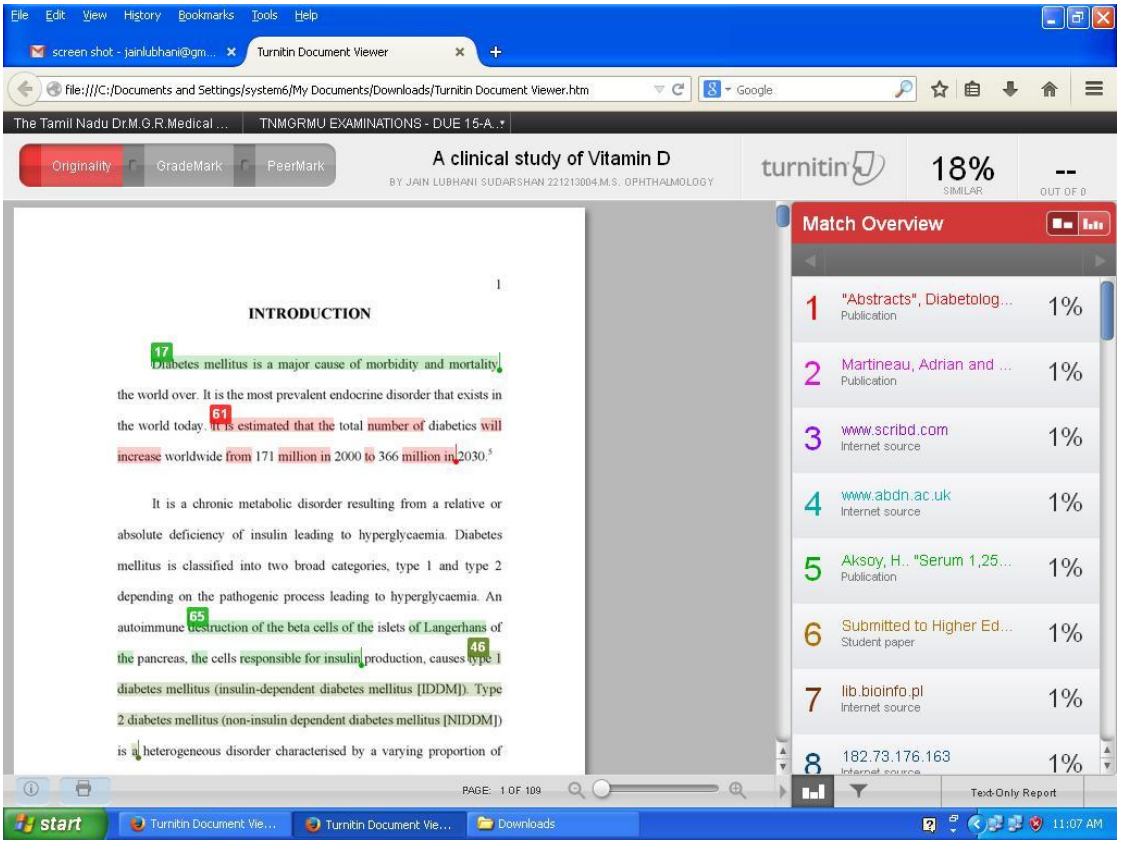
We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

Vice-Principal
Madras Medical College
Chennai-600 003.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INTRODUCTION
Diabetes mellitus is a major cause of morbidity and mortality the world over. It is the most prevalent endocrine disorder that exists in the world today. It is estimated that the total number of diabetics will increase worldwide from 171 million in 2000 to 366 million in 2030.[5]
It is a chronic metabolic disorder resulting from a relative or absolute deficiency of insulin leading to hyperglycaemia. Diabetes mellitus is classified into two broad categories, type 1 and type 2 depending on the pathogenic process leading to hyperglycaemia. An autoimmune destruction of the beta cells of the islets of Langerhans of the pancreas, the cells responsible for insulin production, causes type 1 diabetes mellitus (insulin-dependent diabetes mellitus [IDDM]). Type 2 diabetes mellitus (non-insulin dependent diabetes mellitus [NIDDM]) is a heterogeneous disorder characterised by a varying proportion of
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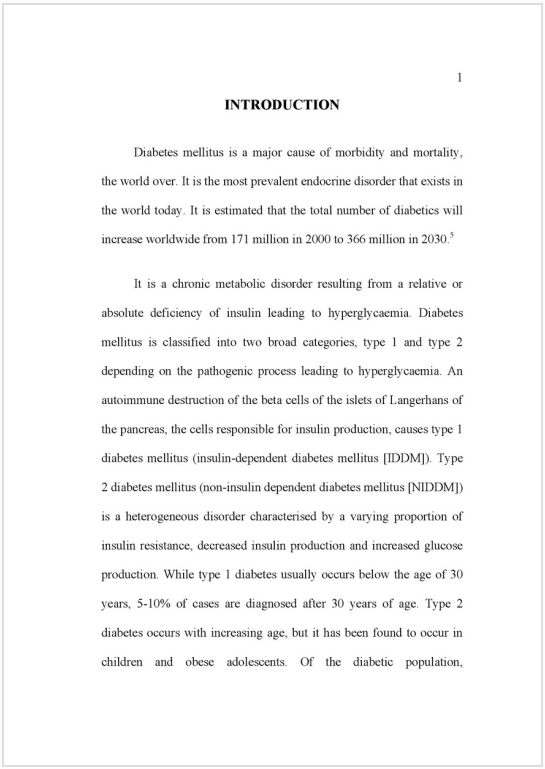

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**A CLINICAL STUDY OF VITAMIN D SUPPLEMENTATION IN DIABETIC RETINOPATHY PATIENTS WITH TYPE 2 DIABETES MELLITUS**" is a bonafide and genuine research work carried out by me under the guidance of **PROF. DR. P.S. MAHESWARI., M.S. D.O.**

DATE:

PLACE: **DR. JAIN LUBHANI SUDARSHAN**

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ABBREVIATIONS

PDR	–	Proliferative diabetic retinopathy
NPDR	–	Non proliferative diabetic retinopathy
IDDM	–	Insulin dependent diabetes mellitus
NIDDM	–	Non insulin dependent diabetes mellitus
FBS	–	Fasting blood glucose
PPBS	–	Post prandial blood glucose
HbA1c	–	Glycosylated haemoglobin
VEGF	–	Vascular endothelial growth factor
NVD	–	New vessels on the disc
NVE	–	New vessels elsewhere
CSME	–	Clinically significant macular edema
FFA	–	Fundus fluorescein angiography
OCT	–	Optical Coherence tomography
CMT	–	Central macular thickness
BCVA	–	Best corrected visual acuity
OHA	–	Oral hypoglycaemic agents
25(OH)D	–	25 hydroxy Vitamin D
IOP	–	Intraocular pressure

INTRODUCTION

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approximately 10% have type 1 diabetes, a majority comprising of type 2 diabetes.

Diabetes is known to cause an array of microvascular and macrovascular complications such as nephropathy, neuropathy, coronary artery disease, peripheral vascular disease and cerebrovascular disease. Among the ophthalmic complications are corneal abnormalities, iris neovascularisation, glaucoma and cataracts. However the commonest ophthalmic complication remains diabetic retinopathy, a sight threatening disorder, which is its microvascular complication. It may have a devastating impact on an individual's quality of life, and hence early detection through routine screening for patients at risk for developing diabetic retinopathy and those who may progress to a severe stage is essential to reduce preventable blindness from this disease.

Diabetic retinopathy, in general, has been observed to progress through the stages of mild to moderate and severe non-proliferative diabetic retinopathy, in an orderly fashion, before the development of proliferative diabetic retinopathy and its vision-threatening complications such as vitreous haemorrhage and tractional retinal detachment. Several factors are known to influence the severity and

progression of diabetic retinopathy such as the duration of diabetes, glycaemia control, blood pressure, serum creatinine concentration and the body mass index.

Vitamin D, until recently was known for its effects on the musculoskeletal system, however various studies have now demonstrated the non-traditional benefits of Vitamin D, including its role in diabetes mellitus. Chiu KC et. Al. in 2004 showed that it is required for normal insulin secretion and glucose homeostasis.[6] It has been shown to have anti-inflammatory, antioxidant, antiangiogenic and antiproliferative functions.

Vitamin D deficiency is a growing health care concern. Several studies have demonstrated widespread vitamin D deficiency and insufficiency, both in patients with a wide spectrum of diseases and in apparently healthy individuals. Holick, M.F. in 2007 found that an estimated 1 billion people were affected by vitamin D deficiency.[7]

The role of vitamin D on insulin secretion and sensitivity has been established by Norman A. W. et. Al. in 1980, Chertow B.S. et. Al. in 1983 and Cade C. et. Al. in 1986, leading to a proposal that it may influence the occurrence as well as treatment of diabetes.[8,9,10] A

reduced risk of development of diabetes has been demonstrated in patients on vitamin D supplementation has been demonstrated by Pittas et al.[11] These results suggested that improving vitamin D status in patients with type 2 diabetes may play a role in controlling the micro-vascular complications of diabetes, namely diabetic retinopathy.

REVIEW OF LITERATURE

Diabetic retinopathy is a distressing microvascular complication of both type 1 and type 2 diabetes mellitus. Damage observed in diabetic retinopathy is through the formation of advanced glycosylation end products and increased metabolism through the sorbitol and hexosamine pathway leading to increased production of several growth factors such as vascular endothelial growth factor (VEGF), thereby aggravating the disease process.

The cause of loss of vision in diabetic retinopathy may be macular oedema, macular ischemia, vitreous haemorrhage and tractional retinal detachment. In patients with background diabetic retinopathy, the progression to vision threatening diabetic retinopathy at 1 year has been estimated to be 5% and for pre-proliferative retinopathy the same has been found to be 15%.

According to the WHO publication of 2004, there are about 31.7 million patients suffering from diabetes in India, resulting in the highest ranking worldwide. This number is predicted to progress to 79.4 million by 2030.

The Chennai Urban Rural Epidemiology Study (CURES) I demonstrated the prevalence of diabetic retinopathy as 17.6%. The Chennai Urban Rural Epidemiology Study (CURES) 2 study was undertaken to demonstrate the association between diabetic retinopathy and serum lipids in South Indian urban population.[12]

Early Treatment Diabetic Retinopathy Study (ETDRS) was a multicentric, randomised clinical trial. It evaluated the role of argon laser photocoagulation and aspirin treatment in the management of non-proliferative or early proliferative diabetic retinopathy.[13] The ETDRS group proposed the ETDRS classification which is the modified Airlie House classification of diabetic retinopathy and was used in the DRS.[72] It classified diabetic retinopathy into mild, moderate, severe, very severe, early PDR and high risk PDR based upon the characteristic lesions observed. Seven standard photographic fields were used, with retention of the original standard, colour, non-simultaneous stereoscopic photographs. This classification was more standardised and elaborative and reduced examiner bias.

HISTORY

Retinal manifestation of diabetes were first observed by Eduard Jaeger in 1856. This was possible only after the development of the direct ophthalmoscope in 1855. Albrecht Von Graefe apposed Jaeger's findings, stating that there was no causal relationship between diabetes and retinopathy.

In 1872, Edward Nettleship provided the first histopathological evidence of "Cystoid Degeneration of the Macula" in patients with Diabetes in his publication. The proliferative changes occurring in Diabetic Retinopathy and the importance of tractional retinal detachments and vitreous haemorrhages were then described by Wilhelm Manz in 1876.

However during the early 20th century, the controversy remained regarding whether macular changes caused by diabetes or hypertension and arteriosclerosis. Arthur James Ballantyne in Glasgow, in the late 20th century, provided further evidence to support the fact that diabetes was the cause for the retinopathy observed in these patients.

ANATOMY

The retina forms the inner coat of the eye ball, where the optical image is formed by the optical system of the eye. It is has a purplish red appearance due to the presence of visual purple in the rods. Its thickness ranges from 0.56mm in the peripapillary retina to 0.18 to 0.2 at the equator and about 0.1mm at the ora serrata. It is thinnest at the fovea. It has a surface area of 266 mm^2.

It has an outer pigmented and inner neurosensory layer, both of which are derived embryologically from the neuroectoderm. The outer retinal pigment epithelium develops form the outer layer of the optic cup and the inner neurosensory layer develops from the inner layer of the optic cup. The neural retina terminates anteriorly at the ora serrata.

Specialised Areas of the Neural Retina:

Macula Lutea

It is an oval, yellowish area at the posterior pole of the retina, lying temporal to the optic disc. It measures about 5.5 mm in diameter.

Fovea Centralis

It is a depressed area in the centre of the macula lutea, measuring about 1.85mm in diameter. The floor is called the foveola and the sides of the depression are called Clivus. This depressed area is formed due to the nerve cells being displaced peripherally, leaving only the cones in the centre. There are no rods or blood vessels overlying the fovea. The fovea has the highest concentration of cones and hence represents the area of maximum visual acuity of the eye. It accounts for 5 degrees of the visual fields.

The foveola is located 2 disc diameters from the temporal edge of the optic disc and 1 mm below the horizontal meridian. It measures 0.35 mm in diameter. The umbo is a depression at the centre of the foveola and is responsible for the foveolar reflex. The foveal avascular zone is situated outside the foveola, but inside the fovea.

Optic Disc

It is about 1.5 mm in diameter. All the retinal layers except the nerve fibre layer end here. It is insensitive to light due to the absence of photoreceptors and is hence called the blind spot. It has a central

depression which is the physiological cup from where the central retinal vessels enter and leave the eye.

Histology

The retina is composed of 10 layers, based on light microscopic findings. These, from outside to inside are:

1. The retinal pigment epithelium
2. The rods and cones
3. The external limiting membrane
4. The outer nuclear layer
5. The outer plexiform layer
6. The inner nuclear layer
7. The inner plexiform layer
8. The ganglion cell layer
9. The Nerve fibre layer
10. The internal limiting membrane

Blood Supply

It is from 2 sources:

1. The Outer Lamina consisting of four layers namely the pigment epithelium, the layer of rods and cones, the external limiting membrane and the outer nuclear layer are supplied by the choriocapillaries.

2. The Inner Lamina comprising of the remaining 6 layers namely the outer plexiform layer, inner nuclear layer, ganglion cell layer, nerve fibre layer and the internal limiting membrane is supplied by the central retinal artery and veins.

3. The outer plexiform layer is supplied partially by the choriocapillaries and partially by the central retinal artery.

Blood Retinal Barrier

The normal retinal capillaries are lined by endothelial cells which are bound together by intercellular junctions of the zonula occludens type. The free flow of solutes and fluids from the retinal vasculatures into the interstitium is prevented by these junctions hence

constituting the blood retinal barrier. These endothelial cells are surrounded by a basement membrane and pericytes. In diabetes, this blood retinal barrier has been found to be compromised leading to the characteristic changes observed in diabetic retinopathy.

EPIDEMIOLOGY

There are 171 million people worldwide suffering from diabetes mellitus, a figure which is predicted to be doubled by 2030, according to the 2004 WHO publication. In 2002, 5 million people were blind due to diabetic retinopathy, constituting 5% of the world blindness. The prevalence of diabetic retinopathy in India is found to vary between 10.5% to 26.2%. It is a leading cause of new blindness for individuals between 20 to 74 years of age in both developed and developing countries. This increasing incidence has lead to its inclusion in the "priority list" of "Vision 2020".

Type 1 diabetes mellitus (IDDM) comprises of 10-15% of all diabetics, the remaining 85% being type 2 diabetes mellitus (NIDDM). In type 1 diabetes of less than 5 years duration, diabetic retinopathy is seen in 13% of patients, which increases to 90% with a duration of 10-15 years and 100% with a duration of greater than 20 years.

In NIDDM there is an increased occurrence of diabetic retinopathy with increasing duration of diabetes. For patients with duration of greater than 5 years, it has been found to have an estimated

prevalence of 40% in those taking insulin and 24% in those not taking insulin.

While patients with Type 1 diabetes have a high incidence of diabetic retinopathy, type 2 diabetes still accounts for the causation of a majority of the cases observed, due to its increased prevalence.

The prevalence of non-proliferative retinopathy, proliferative retinopathy and macular oedema was reported as 71%, 23% and 11% in IDDM and 47%, 6% and 8% in NIDDM respectively, in the WESDR. They reported a 10 year incidence of retinopathy in IDDM below 30 years age as 89% and that above 30 years age as 79%. In NIDDM patients greater the 30 years age the incidence of retinopathy is less in the estimates provided by more recent population-based studies.[14,15]

A more recent study which compiled data from a total of other eight studies including WESDR, was an effort to provide a more accurate estimate of the prevalence of retinopathy in patients who were 40 years or older.[16] The estimates of retinopathy were found to be lower than in the WESDR study. All of these studies were performed at least 10 years after the WESDR study. This study stated that among

the diabetics the crude prevalence of retinopathy was 40%, while 8% of diabetic patients suffered from visual loss due to retinopathy.

Due to advances in the management of diabetes, the prevalence of diabetic retinopathy and its consequential visual loss has not been found to increase, accompanying the increased prevalence of diabetes which has been noted in the recent years. This is attributed to a more stringent glycaemia control in patients with IDDM. This is reflected by the fact that up to 85% patients were found to be using more than 3 injections of insulin daily and up to 91% of patients were practising self monitoring of blood glucose. This resulted in a decrease in the mean levels of HbA1c to 7.6% and up to 33% diabetics attaining the ADA guidelines of a glycosylated haemoglobin level of less than 7%, as observed in the WESDR.

Following the UKPDS study there was an increase in the management of NIDDM using multiple oral hypoglycaemic agents, as opposed to the use of only one such drug prior to this study. This resulted in a reduction of the mean HbA1c levels from 7.8% to 7.2% and a 41% increase in patients achieving mean HbA1c levels of less than 7%.[17]

Factors that may increase the incidence of diabetic retinopathy include hypertension, obesity, hyperlipidemia, pregnancy and oral contraceptives, glomerulosclerosis and a sudden shift from oral hypoglycaemic agents to insulin.

FACTORS INFLUENCING DIABETIC RETINOPATHY

1. Duration of diabetes

This is one of the most consistent risk factors for the development and increase in severity of diabetic retinopathy. It is a significant risk factor for the development of maculopathy. In IDDM, retinopathy develops at least 3-4 years after disease onset. PDR was found in 33% and 50% of women and men respectively following 20 year duration of diabetes. Since the duration of development of type 2 diabetes is difficult to determine, retinopathy may present as an initial sign soon after diagnosis. 20 years after the development of diabetes, 100% of patients with IDDM and 60% of patients with NIDDM were found to have developed proliferative diabetic retinopathy. In both type 1 and type 2 diabetes duration of diabetes as a factor for prevalence of macular oedema was identical.[15]

2. Age

An increase in the prevalence of retinopathy has been observed as age advances. The hormonal changes during puberty influence the progression of retinopathy. Elevated Insulin like Growth Factor I (IGF I) is produced under the influence of growth hormone and closely resembles a chain of insulin. It is known to enhance the development of diabetic retinopathy.

3. Sex incidence

An increased incidence of proliferative diabetic retinopathy was observed in men with an earlier onset of diabetes than women, in the WESDR study. However besides this, no significant difference in the prevalence or progression of retinopathy was observed between males and females in the WESDR study.

4. Race

Blacks have a 20% higher incidence then whites. Several studies have demonstrated that the prevalence of retinopathy in NIDDM was greater in African Americans.[18,19,20,21] In the NHANES (1988-94 and 2005-8) and the MESA, retinopathy was more frequent in Mexican

Americans then the non-Hispanic whites who were 40 years of age or older.

5. Genetic factors

Data from several studies has revealed familial clustering of diabetic retinopathy suggesting a genetic susceptibility. Patients with HLA DR 4 and DR 5 phenotype have been found to have an increased risk of proliferative diabetic retinopathy. HLA B 15 individuals are more likely to develop diabetic retinopathy, while individuals with HLA B 7 are 4 times less likely to develop PDR.

6. Glycaemic control

Retrospective studies measuring glycosylated haemoglobin, evaluating long term control of blood glucose have suggested that hyperglycaemia is closely associated with the development of retinopathy.

According to the Diabetic Control and Complications Trial (DCCT), a 10% decrease in HbA1c (from 8% to 7.2%) results in a decrease in incidence of diabetic retinopathy by 35% to 40%. The results of the DCCT are as follows:

- Intensive therapy reduced the first appearance of any retinopathy by 27% and clinically meaningful diabetic retinopathy by 35 to 74%.

- Intensive therapy also reduced the development of other microvascular complications such as reduction of microalbuminuria by 35%, clinical proteinuria by 56% and clinical neuropathy by 60%.

- Intensive treatment included insulin administration at least thrice daily with dose adjustment on the basis of self blood glucose monitoring with aim of achieving normoglycaemia.

However this study was based on the results obtained from patients suffering from IDDM and not NIDDM.[22]

On the other hand UKPDS, conducted between 1977 to 1991, included only NIDDM patients. It evaluated glycaemia control with either insulin or sulphonylurea. The study concluded that intensive blood glucose control reduced the progression of retinopathy and the development of other microvascular complications of diabetes.

These studies conclusively demonstrate that stringent control of blood glucose, both in patients with IDDM and NIDDM, is of utmost importance to decrease the sight threatening complications resulting from retinopathy. The studies further emphasized the importance attaining the ADA guidelines of a target of HbA1c levels below 7% and suggest that the earlier this level is achieved after the diagnosis of diabetes, the lower the risk for development and progression of retinopathy.

7. Hypertension

Increased blood pressure levels enhance the risk of development and progression of retinopathy. A similar association has been found between an increase in diastolic blood pressure and a rise in the incidence of diabetic macular oedema. The UKPDS demonstrated on reducing the systolic blood pressure, decrease in the incidence of microvascular complications of diabetes including retinopathy was observed.

8. Pregnancy

Pregnancy creates a state of insulin resistance thereby promoting the progression of retinopathy.

9. Protienuria and diabetic nephropathy

Diabetic nephropathy has been found to be a significant risk factor in the development and progression of diabetic retinopathy.

10. Serum lipids

In the ETDRS, higher levels of serum lipids at baseline were associated with an increased incidence of hard exudates at the macula and decreased visual acuity. Patients with hypercholesterolemia and receiving insulin therapy, demonstrated an increase in the number of hard exudates on fundus evaluation. [14,15]

11. Smoking

It has been suggested that smoking could aggravate retinal hypoxia by promoting aggregation of platelets and causing vasoconstriction of retinal vessels as a result of increased carbon

monoxide concentrations. However most studies, showed no association between smoking and retinopathy.[14,15]

12. Alcohol

In patients with IDDM, alcohol intake was found to decrease the incidence of PDR in some studies.[14,15] In the UKPDS, an increased alcohol consumption was found to be related to an increased incidence of retinopathy only in newly diagnosed young men with NIDDM.

13. Ocular factors

Glaucoma, myopia and retino-choroidal scarring from trauma and inflammation have been proposed to protective against the development of retinopathy.

CLASSIFICATION

The most wide used classification internationally is that proposed by the Early Treatment Diabetic Retinopathy Study (ETDRS) or the modified Airlie House Classification. It gives a better understanding regarding the progression and management of the disease.

ETDRS Classification of diabetic retinopathy

I. NPDR (Non Proliferative Diabetic Retinopathy)

It is characterised by configurational changes in the veins, exudates and retinal haemorrhages. The severity of retinopathy is sub-classified as

a. Mild NPDR:

   At least one microaneurysms and definition not met for moderate NPDR.

   These patients have a 5 % risk of progressing to PDR within 1 year and a 15 % risk of progressing to high-risk PDR within 5 years.

b. Moderate NPDR:

- Severe retinal haemorrhages (more than ETDRS standard photograph 2A: about 20 medium-large per quadrant) in 1-3 quadrants or mild intraretinal microvascular abnormalities (IRMA).
- Significant venous beading can be present in no more than 1 quadrant.
- Cotton wool spots are commonly present.

The risk of progression to PDR is 12-27 % within 1 year and 33 % within 5 years.

c. Severe NPDR:

The 4-2-1 rule; one or more of:

- Severe haemorrhages in all 4 quadrants
- Significant venous beading in 2 or more quadrants
- Moderate IRMA in 1 or more quadrants

The risk of developing PDR is 52 % within 1 year and 60 % within 5 years.

d. Very severe NPDR

Two or more of the criteria for severe NPDR.

The risk of developing PDR is 75 % within 1 year.

II. Proliferative diabetic retinopathy (PDR)

a. Early

New vessels on the disc (NVD) or new vessels elsewhere (NVE), but extent insufficient to meet the high-risk criteria.

The risk of developing high-risk PDR within 5 years is 75 %.

b. High-risk

- New vessels on the disc (NVD) greater than ETDRS standard photograph 10A (about ½ disc area)
- Any NVD with vitreous or preretinal haemorrhage
- NVE greater than ½ disc area with vitreous or preretinal haemorrhage (or haemorrhage with presumed obscured NVD/E)

Macular oedema can be classified as:

- Focal
- Diffuse

- Ischemic
- Mixed

Clinically significant macular oedema (CSME) was defined in ETDRS as:

- Retinal thickening within 500 microns of the centre of the macula
- Exudates within 500 microns of the centre of the macula, if associated with retinal thickening (which may be outside the 500 microns)
- Retinal thickening one disc area (1500 microns) or larger, any part of which is within one disc diameter of the centre of the macula.

FIG.1 - MILD NON PROLIFERATIVE DIABETIC RETINOPATHY

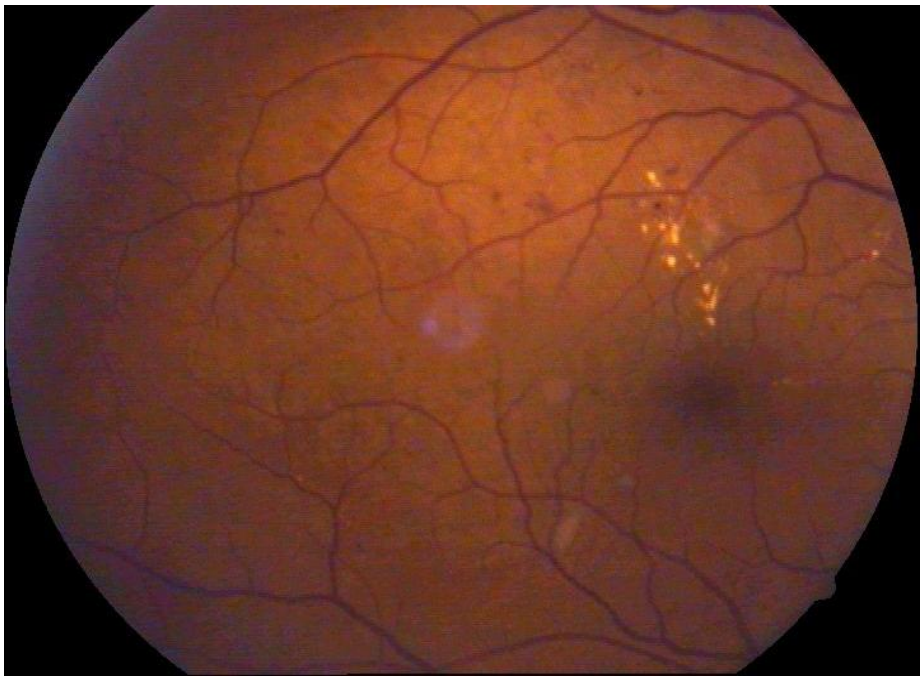


FIG.2 - MODERATE NON PROLIFERATIVE DIABETIC RETINOPATHY

FIG.3 - SEVERE NON PROLIFERATIVE DIABETIC RETINOPATHY

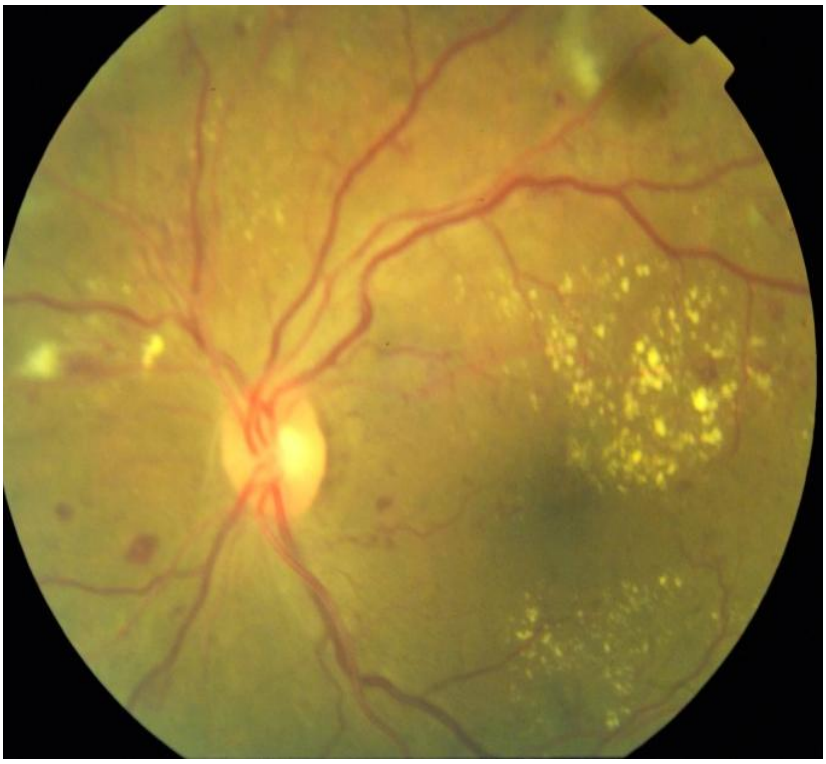


FIG.4 - VERY SEVERE NON PROLIFERATIVE DIABETIC RETINOPATHY

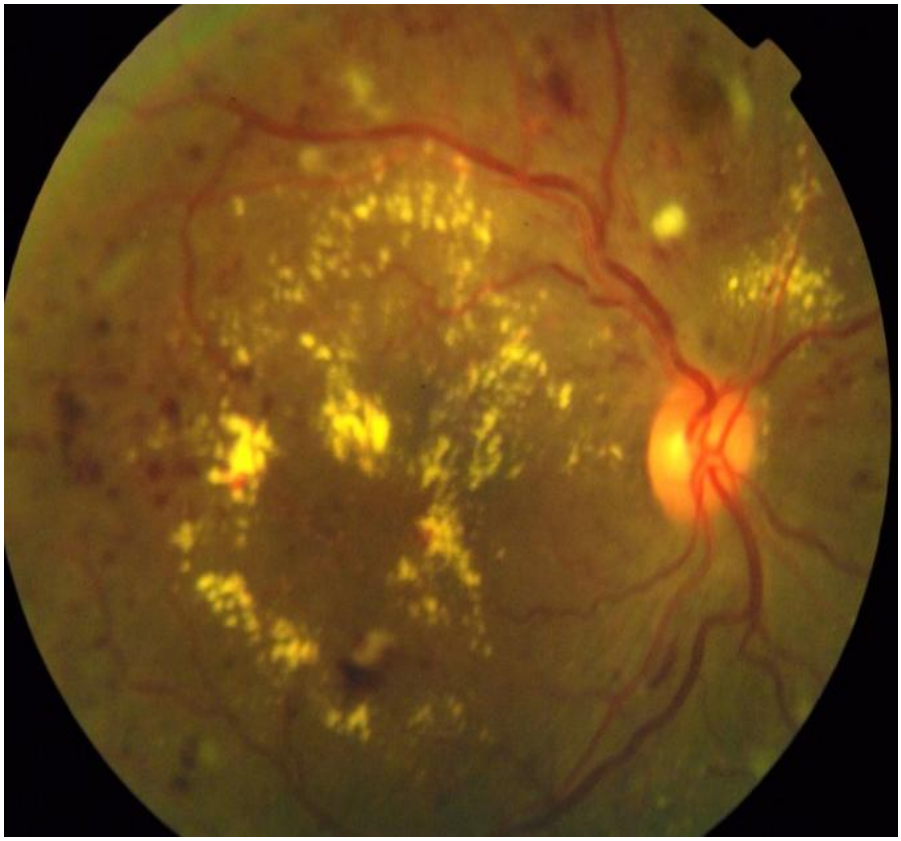


FIG.5 - FLORID NEW VESSELS ON THE DISC (NVD) IN HIGH RISK PROLIFERATIVE DIABETIC RETINOPATHY

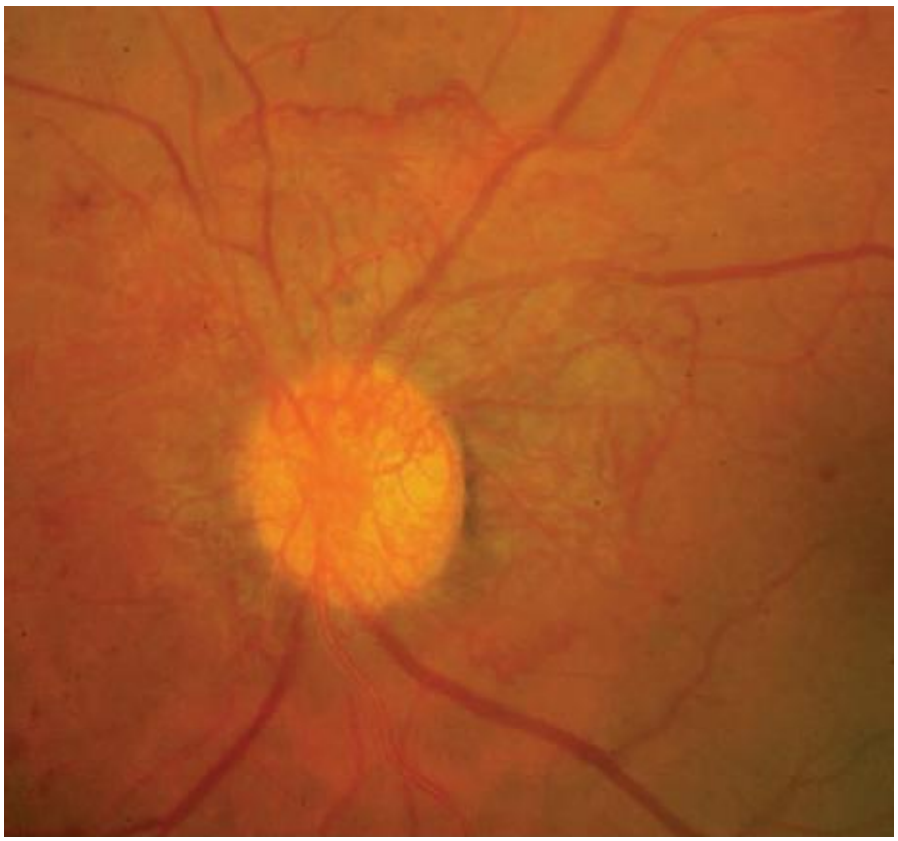


FIG.6 - PRE-RETINAL HAEMORRHAGE WITH NEW VESSELS ELSEWHERE (NVE)

FIG.7 - MACULAR SUB HYALOID HAEMORRHAGE

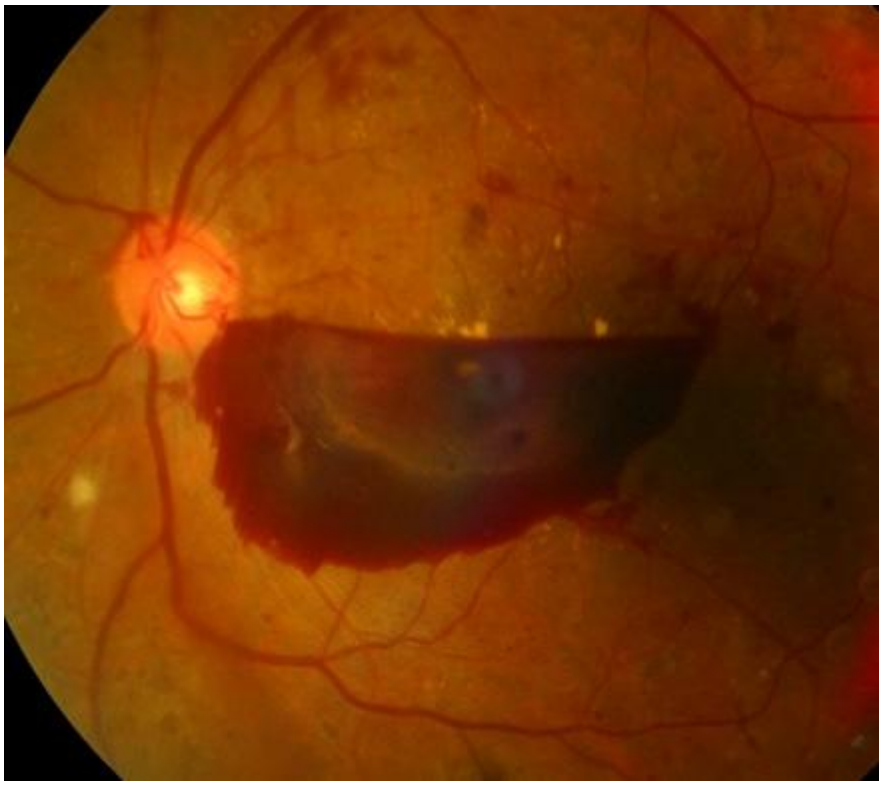


FIG.8 - VITREOUS HAEMORRHAGE

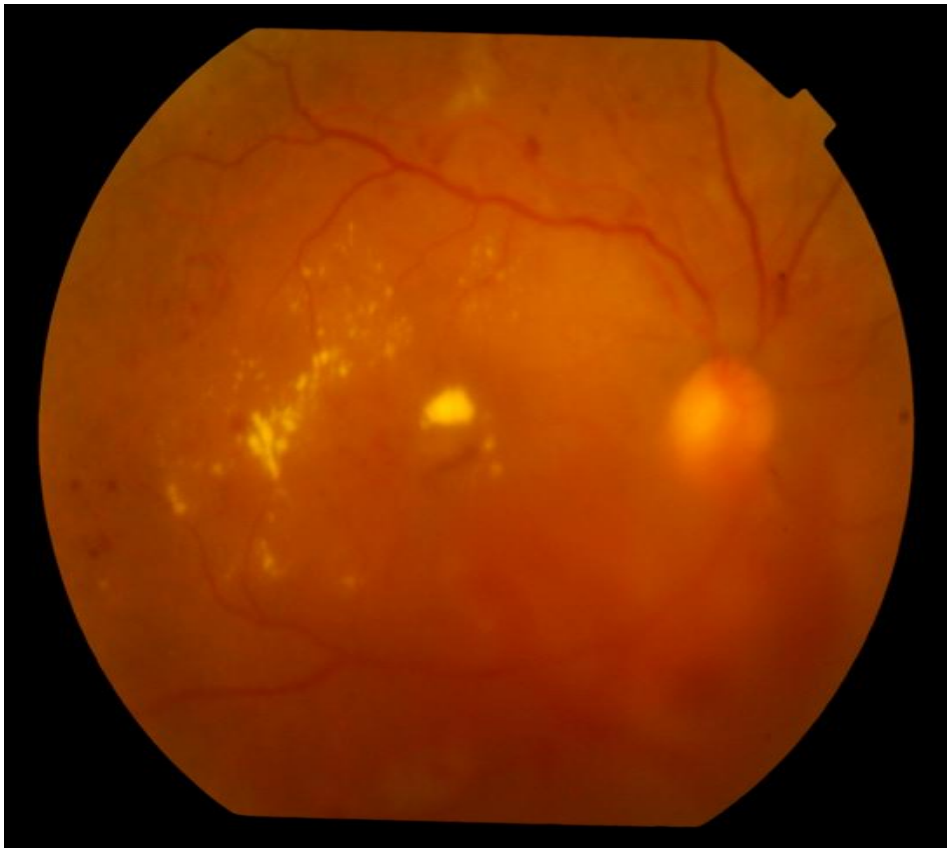
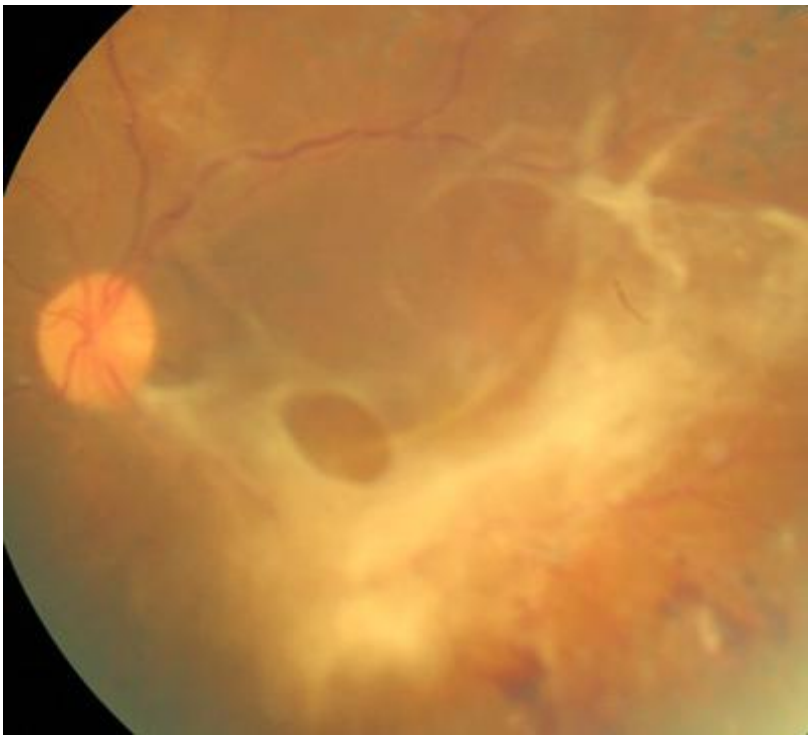


FIG.9 - TRACTIONAL RETINAL DETACHMENT



PATHOGENESIS OF DIABETIC RETINOPATHY

Structural, rheological and biochemical factors contribute to the development of diabetic retinopathy.

I) Structural changes

1. Capillary basement membrane thickening

Electron microscopy has proved marked thickening of the basement membrane, with swiss-cheese like vacuolization and deposition of fibrillar collagen which stains positive for type III collagen.

2. Loss of microvascular intramural pericytes

In digest preparation they have been noted as empty, balloon like spaces bulging from the side of the capillary wall. This is probably related to the action of the sorbitol pathway.

3. Loss of endothelial cells and endothelial cell dysfunction

FFA has demonstrated that acellular capillaries are non-functional and appear as dark spaces on the angiogram. The endothelial

cell junctions become loose which could be related to the reduced expression of ZO-1 protein. Fenestrations appear in the endothelial cell cytoplasm.

II) Rheological changes

1. Platelet abnormalities

An increased platelet adhesiveness and aggregation and decreased platelet survival has been demonstrated by various studies. The cause of increased platelet aggregation may be due to elevated levels of Von Willebrand factor-Factor VIII, increased production of thrombaxane A2 by platelets and reduced prostaglandin production by endothelial cells

2. Red blood cell abnormalities

A decreased deformability and increased formation of rouleaux aggregates has been shown. A multitude of factors contribute to local ischemia probably due to increased red cell aggregation such as increased level of fibrinogen and alpha 2 globulin, inhibition of plasmin by alpha 2 globulin and diminished fibrinolytic response. The

increased erythrocyte aggregation results in increased blood viscosity and microinfarction of the retinal vasculature.

III) Biochemical changes

1. Prolonged hyperglycaemia

This is a major etiological agent for all microvascular abnormalities of diabetes including retinopathy. The cellular mechanisms postulated are as follows:

- Prolonged hyperglycaemia alters the expression of genes leading to the formation of gene products that can alter cellular functions
- Advanced glycation end products are formed by the non-enzymatic binding of several sugars to proteins, which are long lived and play a causal role in diabetic complications
- Chronic hyperglycaemia may produce accelerated oxidative stress in cells leading to increased formation of toxic end products.

2. Sorbitol pathway

It is the most prominent enzymatic mechanism suggested to cause diabetic retinopathy and other complications. Sorbitol formed from glucose by aldose reductase, is slowly converted by sorbitol reductase to fructose. Since the latter reaction is a slow process, sorbitol accumulates in toxic concentrations leading to endothelial cell damage and cellular oedema.

3. Diacyl glycerol (DAG) and Protein Kinase C (PKC)

The level of DAG and the activity of PKC is elevated in patients with diabetes leading to decreased retinal blood flow.

4. Insulin receptors and glucose transportation

In quantitative immunohistochemistry studies, GLUT 1 (glucose transporter 1) immunoreactivity was present in the retina of half the patients suffering from diabetes, however the implications of this finding remain unclear.

5. Vascular endothelial growth factor (VEGF)

It is an angiogenic factor. An upregulation of genes for its expression has been noted in hypoxia of proliferative diabetic retinopathy. It may also be responsible for macular oedema in diabetic retinopathy.

Hence the following sequence of events can be postulated in diabetic retinopathy:

1. Hyperglycaemia with insufficient insulin is the starting point. Advanced glycation end products are formed due to nonenzymatic binding of several sugars to proteins. Chronic hyperglycaemic results in oxidative stress.

2. Altered signalling of pathways involving protein kinase C, nuclear factor kappa-B and MAP kinase occurs due to increased polyol metabolism of glucose.

3. This results in damage to RPE cells, endothelial cells, pericytes and neurons. Recruitment of inflammatory cells also occurs.

4. Elevated growth hormone and reduced insulin levels alter the hepatic cell protein synthesis leading to dysproteinaemia.

5. Elevated fibrinogen and alpha 2 globulin result in increased red cell aggregation

6. Elevated growth hormone is associated with increased production of Von Willebrand factor and reduced prostacyclin levels leading to increased platelet aggregation.

7. Hyperglycaemia impairs prostacyclin production by endothelial cells.

8. Impaired RBC and platelet aggregation impairs haemorheodynamics in the microcirculation.

9. Impaired blood flow in the microcirculation caused by a sluggish flow rate or microinfarction leads to hypoxia and ischemia.

10. Release of vasoactive compounds, coagulation factors, growth factors and adhesion molecules occurs leading to angiogenesis and tissue remodelling, breakdown and leakage of retinal vessels and transudation and exudation of blood elements into the retinal structure.

CLINICAL FEATURES

Diabetic retinopathy can be broadly classified into the proliferative and non-proliferative varieties. The hallmark of proliferative retinopathy being presence of new vessels in the retina and optic disc. The clinical features are as follows:

1. Microaneurysms

These are usually the first visible signs of diabetic reinopathy and the hallmark of NPDR. They are identified ophthalmoscopically as red dots, most commonly in the posterior pole. Their size ranges from 15 to 60 microns in diameter. Histologically, they are hypercellular outpouchings of the capillary wall. On FFA, they can be identified as hyperfluorescence dots much more extensive than those identified clinically. Punctuate haemorrhages, in contrast show blocked fluorescence on FFA. Hence FFA can be used to distinguish between the two.

2. Hard exudates-

Hard exudates are small white or yellowish-white deposits with sharp margins. They are formed due to lipid extravasation and are

usually seen as discrete intra retinal deposits surrounding microaneurysms in a circinate ring. They are usually located in the outer layers of retina, but can be more superficial and may be seen as individual dots or confluent patches. In late stages subretinal exudates can be replaced by fibrotic plaques.

3. Soft exudates-

These are small, whitish, fluffy, superficial lesions that represent infarction of the retinal nerve fibre layer with accumulation of neuronal debris. The swollen ends of the disrupted nerve axons are known as cystoids bodies.

4. Intraretinal Microvascular Abnormalities(IRMA)-

These are arteriovenous shunts that run from retinal arterioles to venules, bypassing the capillary bed. Hence they are seen in areas adjacent to marked capillary non-perfusion as fine, red, irregular intraretinal lines.

5. Venous abnormalities-

Venous changes observed include dilatation and tortuosity, sausage like segmentation, looping and beading consisting of focal

narrowing and dilatation. Abnormalities within one half disc diameter of the disc margin are ignored. These changes correlate with risk of developing proliferative changes.

6. Arteriolar abnormalities-

These are early markers of ischemic changes and include peripheral narrowing, silver-wiring and vascular obliteration. Abnormalities within one half disc diameter of the disc margin are ignored.

7. Capillary closure

It is one of the most important features of diabetic retinopathy, the extent and location of which is most accurately visualised on fluorescein angiography.

8. Retinal haemorrhages

They may be superficial or deep and are seen predominantly on the posterior pole. They may disappear only to reappear in a different area of the retina. Dot and blot haemorrhages are located within the compact deeper layers of the retina and result from venous

engorgement of capillaries. Deeper dark round haemorrhages representing hemorrhagic retinal infarcts may also occur. These may indicate subsequent progression to neovascularisation. Flame shaped haemorrhages arise in the nerve fibre layer from pre-capillary arterioles.

9. Preretinal haemorrhage-

Both boat shaped haemorrhages with a fluid level and round, oval or linear patches of the haemorrhage just anterior to the retina or under its internal limiting membrane are included in pre-retinal haemorrhages. Haemorrhages on the surface of the detached retina, is also considered to be preretinal haemorrhage.

10. Diabetic macular oedema

Localised oedema is caused by focal leakage from dilated capillary segments or microaneurysms, while diffuse retinal oedema is caused by extensive capillary leakage. Lipid may also escape into the extravascular space and collect within the retina forming hard exudates. The fluid initially accumulates between the inner nuclear

layer and the outer plexiform layer, however it may eventually involve the entire thickness of the retina.

11. New vessels-

These are most commonly seen at the posterior pole, though they may occur anywhere in the posterior pole. At least one quarter of the retina must be non-perfused prior to the development of proliferative changes. They appear as irregular vessels, often forming networks which may be accompanied by fibrous tissue which gradually increases as these vessels increase in size. Neovascularisation at the disc (NVD) is neovascularisation on or within one disc diameter of the optic disc, while neovascularisation elsewhere (NVE) is present away from the optic disc. Neovascularisation of the iris (NVI) may also develop which has a high risk for the development of neovascular glaucoma.

12. Vitreous changes

Posterior vitreous detachment may occur. New vessels on the retina are adherent to the posterior vitreous surface. As the vitreous starts separating, these vessels are stretched and may result in bleeding

into the vitreous cavity. Pre-retinal haemorrhages may also break into the vitreous. Vitreous condensation and fibrosis may also occur.

13. Retinal detachment

Tractional retinal detachment may occur, the extent and location of which depends on vitreo-retinal attachments.

CLINICAL EVALUATION

1. Visual Acuity

Visual loss mainly depends on the involvement of the macula.

2. Indirect Ophthalmoscopy and slit lamp biomicroscopy with 90 D lens

This technique allows the examiner to integrate the view of the entire retina.

3. Direct Ophthalmoscopy

Though the area of retina examined is smaller, the increased magnification of this method allows detailed examination of the various retinal lesions.

4. Fundus Photography

The ETDRS classification used 7 standard photographic fields including one each centered on the disc, centered on the macula, temporal to the macula so that its nasal edge passes through the centre of the macula and the remaining four tangent to the vertical line

passing through the centre of the disc and horizontal lines passing through its upper and lower borders. It is useful for documentation of lesions and during follow up.

5. Fundus Fluorescein Angiography

This is one of the essential investigations needed in diabetic retinopathy. It can demonstrate microaneurysms, intraretinal microvascular abnormalities, new vessels and the extent of capillary closure. It provides the earliest evidence of a breech in the blood retinal barrier. Macular oedema can also be detected on FFA and classified into the focal, diffuse or ischemic variety. In eyes with asteroid hyalosis or hazy media, FFA may reveal lesions previously undetected on fundus examination.

6. Optical Coherence Tomography

It enables detection and quantification of macular oedema and vitreo-macular traction. The central macular thickness is used routinely to guide the decisions regarding the treatment modalities. The visual acuity may not correlate with OCT findings.

7. Ultrasonography

It is an essential tool in the presence of opaque media to determine the status of the retina. It can detect the presence of retinal detachments, preretinal membranes and vitreous haemorrhage.

MANAGEMENT

I. Non-Proliferative Diabetic Retinopathy

The Diabetic Retinopathy Study (DRS) and the Early Treatment Diabetic Retinopathy Study (ETDRS) are two landmark trials where laser photocoagulation was the principal modality of treatment. The results of these trials form the basis for the guidelines presently used in the treatment of diabetic retinopathy. The treatment for NPDR varies based upon the severity of the diabetic retinopathy and whether there is an associated clinically significant macular oedema.

II. Proliferative diabetic retinopathy

The treatment of PDR aims at achieving two main goals, one being the prevention of neovascular proliferation the other is to release the tractional forces exerted on the retina by the fibrovascular proliferations and posterior vitreous surface contraction. Good glycaemia control and its remarkable efficacy in discouraging the proliferation of new vessels have been proved (DCCT, 1993).

Laser photocoagulation

It involves transpupillary delivery of laser energy for the production of burns in the retina. It causes destruction of some retinal areas, allowing the blood and oxygen to be better delivered to the rest of the retina, thus having a beneficial role in retinal ischemia and decreasing the release of angiogenic factors.

Scatter Photocoagulation for NPDR

It has been observed that the risk-benefit ratio of photocoagulation becomes more favourable as the retinopathy progresses from severe or very severe non proliferative stage or early proliferative stage. The risk of visual loss is significantly reduced by early scatter photocoagulation in patients with NIDDM (Ferris et al).

Photocoagulation for Diabetic Macular Oedema

It is the treatment of choice in clinically significant macular oedema, which may be focal or grid laser photocoagulation. The focal laser photocoagulation used in ETDRS is indicated in cases of focal oedema, where the leaking microaneurysm can be identified. It involves direct application of laser burns to all leaking microaneurysms

in the edematous retina. Laser burns must be of 50 – 100 microns in diameter, 0.1 seconds exposure time and must be applied between 500 – 3000 microns from the centre of the fovea. In ETDRS, the grid laser photocoagulation was used in cases of diffuse macular edema where focal areas of leakage could not be identified. It consists of light intensity burns with a spot size of 50-200 microns, exposure time of 0.1 seconds and spaced more than 1 burn width apart.

The adverse effects of photocoagulation observed in some patients include the development of choroidal neovascularisation, which may lead to subretinal fibrosis. The risk factor for these adverse effects was the presence of extensive hard exudates depositions in the posterior pole in patients with hyperlipidemia.

The ETDRS study demonstrated that treated eyes had a 12 percent loss of 15 letters of vision in 3 years compared to 24 percent in the untreated eyes.

Photocoagulation for PDR

Mayer- Schwickerath developed the xenon arc photocoagulator, which was first used in PDR for the treatment of retinal new vessels.

There has been wide agreement that prompt treatment must be initiated in most eyes with PDR that have well established NVD or vitreous or preretinal haemorrhages. Extensive neovascularisation in the anterior segment is a strong indication for scatter photocoagulation. Cryo applications or vitrectomy with endo photocoagulation are other alternatives which may be used if media opacities prevent visualisation of the retina. Signs of severe retinal ischemia increase the urgency to initiate scatter photo coagulation, as these eyes are more prone to anterior neovascularisation.

ETDRS Protocol for Photocoagulation

The scatter protocol used argon blue green or green laser burns, with a spot size of 500 microns, exposure time of 0.1 seconds and of moderate intensity. The laser burns were applied one half burn width apart, from the posterior pole to the equator. A total of 1200 – 1600 burns must be applied, which may be divided into two or more treatment sessions. Direct treatment was specified for patches of surface NVE that were two disc areas or less in extent. Confluent moderately intense burns were used in such cases that extended 500 microns, beyond the edges of the patch.

The ETDRS recommended that scatter treatment should be considered only in eyes nearing the high risk stage, where the risk-benefit ratio of photocoagulation was approached a favourable range. It must be avoided in mild and moderate NPDR.

In patients with very severe NPDR or moderate PDR, systemic factors must be taken into account while deciding when to begin treatment. Diabetic retinopathy may progress rapidly in pregnancy and renal failure, warranting earlier treatment. Macular oedema sometimes increases at least temporarily, after scatter photocoagulation. This may be followed by transient or persistent reduction of visual acuity.

The six factors to be considered at follow up are -

1. Change in new vessels since the last visit or last photocoagulation.
2. Appearance of new vessels
3. Frequency and extent of vitreous haemorrhages since last visit
4. Status of vitreous detachment
5. Extent of photocoagulation scars
6. Extent of tractional retinal detachment and fibrous proliferation

When additional scatter treatment is carried out using the above mentioned protocol, burns are placed between the treatment scars, sparring the area within 500 microns of the centre of macula using burns not larger than 200 microns. The areas chosen are generally those in which new vessels.

Anti-VEGF drugs

Currently 3 anti-VEGFs are available, Ranibizumab (Lucentis), Pegaptinib (Macugen) and Bevacizumab (Avastin). Vascular endothelial growth factor (VEGF) has been found responsible for most of the proliferative changes in diabetic retinopathy. Hence intravitreal injections of anti-VEGF agents have been postulated to have significant role in treatment.

Several studies have established its role in macular oedema, demonstrating that all 3 drugs were useful in decreasing macular oedema and improving the vision. However, recurrence may occur requiring repeated injections.

Its role in proliferative diabetic retinopathy is still under debate. While some suggested that their use may limit the extent of laser

photocoagulation needed, laser still remains the treatment of choice for PDR. These drugs may be used in persistent neovascularisation not responding to laser therapy and pre-operatively, to reduce the risk of intraoperative haemorrhage.

These drugs may be associated with worsening of fibrosis or precipitation of tractional retinal detachment. Following anti-VEGF injections, a shift in the balance between VEGF and the profibrotic factor, CTGF may occur, promoting these changes.

Steroids

Triamcinolone acetonide was the most common steroid used in the treatment of diabetic macular oedema. Commonly a dose of 4mg is used intravitreally. Although short term results are favourable, reflecting an improvement in the visual acuity and reduction in macular thickness, recurrences are common, requiring repeat injections. This problem has been addressed by Ozurdex, slow release devices, as the frequency of injections is reduced to 6 months or less.

Other medical therapies

Aspirin use did not affect the progression of retinopathy or the risk of visual loss according to the ETDRS. Aldose reductase facilitates the conversion of glucose to sorbitol. The use of aldose reductase inhibitors have not demonstrated any beneficial effects in diabetic retinopathy (Sorbinil Retinopathy Trial Research Group, 1993).[73] Lowering of serum lipids was found useful in macular oedema associated with hyperlipidemia.

Vitrectomy

The DRVS was conducted to explore the possibilities and outcomes of vitrectomy in selected cases. The results suggests that early vitrectomy should be considered in eyes with recurrent vitreous haemorrhage when it is known from prior examination, that fibrovascular proliferation is severe, especially when macular potential is good. Additional indications include non-resolving vitreous haemorrhage, traction on the disc, peripapillary retina or macula that distorts these structures and lead to reduction in vision, tractional retinal detachment, combined tractional and retinal detachment and neovascularisation of iris with hazy media.

SCREENING SCHEDULE - SUGGESTED FOR DETAILED OPHTHALMIC EVALUATION OF DIABETIC PATIENTS

Type of diabetes	Timing of first eye examination	Follow up interval recommended
Type 1	Within 5 years of the diagnosis	Every 1 year
Type 2	At the time of diagnosis	Every 1 year
Any type with pregnancy	Before conception or in the early first trimester	Every 1-3 months in severe NPDR, early PDR or high risk PDR Every 3-13 months in patients with no retinopathy or mild or moderate NPDR

ETDRS RECOMMENDATION FOR FOLLOW-UP

CATEGORY	FOLLOW UP
No diabetic retinopathy	Review in 12 months
Mild NPDR	Review range of 6-12 months, depending stability, severity and associated systemic features
Moderate NPDR	Review in approximately 6 months
Severe NPDR	Review in 4 months
Very severe	Review in 2-3 months
Early PDR	Treatment considered according to stability, severity and associated systemic factors. If the patient is not treated, review in 2 months
High-risk PDR	Treatment should be performed immediately if possible

VITAMIN D AND ITS ROLE IN DIABETIC RETINOPATHY

An increase in the prevalence of vitamin D deficiency has been noted worldwide, along with an increase in the prevalence of diabetes mellitus (Lips P et. Al., Gannage-Yared M-H et. Al.).[23,24] An increased risk of hypovitaminosis D is observed in infants, adolescents and lactating women, which may be attributed to increased requirements during these periods. People living at higher altitudes may also demonstrate lower levels, due to reduced photoconversion of 7-dehydrocholesterol to previtamin D, a problem which is aggravated in winter months as shown by Holick MF et. Al.[25] Mishal AA et. Al. demonstrated that inhabitants of areas with abundant sunlight may also have Vitamin D deficiency due to traditional clothing, exposing little skin to sunlight, resulting in reduced synthesis of vitamin D.[26] A higher incidence of vitamin D deficiency is also noted in south Asian and black populations, due to increased absorption on UV-B resulting from higher levels of skin pigmentation, decreasing vitamin D production. A deficient state can manifest, if the requirements are not compensated by dietary intake. Wortsman J et. Al. showed that reduced physical activity and increased body mass index (BMI) resulting in deposition

of biologically inactive vitamin D in adipose tissue may result in Vitamin D deficiency.[27] Ogunkolade WB et. Al. demonstrated that chewing of beetle nut resulting in increased conversion to inactive vitamin D metabolite, 24,25 (OH)2D is also known to result in a deficient state.[28]

Although Vitamin D is known for its association with calcium and phosphorus homeostasis and bone metabolism, recent evidence has revealed several unconventional functions of vitamin D including its role in respiratory, cardiovascular, infectious and autoimmune diseases and cancer.[29-38]

A role of Vitamin D deficiency in the development of both type 1 and type 2 diabetes mellitus has also been demonstrated.[39-46] An inverse relationship between fasting blood glucose and 25-OHD concentrations was demonstrated by the third National Health and Nutrition Examination Survey (NHANES) data in healthy white postmenopausal women, and in Mexican American men and women, however no such association was seen in non-Hispanic black population, suggesting that ethnicity may also have a role in this effect.[42] The influence of vitamin D on insulin secretion was first demonstrated over 3 decades ago by Norman A.W. et. Al. and was

further supported by animal studies.[47,48] Borrisova A.M. et. Al. and Chiu C.J. et. Al. have now conclusively demonstrated that vitamin D influences the pathogenesis of diabetes mellitus through both pancreatic insulin secretion[49] and insulin sensitivity and thereby affects the pathogenesis of the disease.[50] Hitman G.A. et. Al. demonstrated that the beta cells possess receptors for the activated hormone 1, 25-dihydroxyvitamin D3 (1, 25(OH) 2 D3) and vitamin D-dependent calcium-binding proteins.[51] An improvement in insulin release in response to oral glucose load was seen following Vitamin D supplementation in one study. It was accompanied by a reduction in free fatty acids and an increase in serum calcium.[52] Vitamin D may influence insulin sensitivity by mediating calcium metabolism and regulation of insulin receptor gene.[53] It also regulates human peroxisome proliferator activated receptor D, which may have an important role in insulin sensitivity.[54-55]

Vitamin D has antiangiogenic, antiproliferative, anti-oxidant, immunomodulatory and anti-inflammatory functions in several cells. These functions are mediated by vitamin D receptors, members of the nuclear receptor super family, which is found extensively in the retina.[56] Albert D.M. et. Al. provided supportive evidence for the role

of 1, 25(OH) 2 D3 in inhibiting retinal neovascularization by using a mouse model of ischemic retinopathy,[57] while Kaur H. et. Al. showed that it inhibited endothelial cell proliferation in cell culture studies.[58]

Vitamin D may play a role in development and progression of diabetic retinopathy via inhibition of inflammation, improved insulin secretion and sensitivity, antiproliferative effect on endothelial cells, anti-angiogenic and anti-proliferative actions.

AIMS AND OBJECTIVES

AIMS

To evaluate the effect of supplementation of Vitamin D in delaying the progression, the association of serum 25 hydroxy Vitamin D with the level of Diabetic Retinopathy, and its use as a predictor of the severity of Diabetic Retinopathy.

PRIMARY OBJECTIVE

To evaluate whether the oral supplementation of vitamin D delays the progression of Diabetic Retinopathy.

SECONDARY OBJECTIVE

To evaluate the association between serum 25 hydroxy Vitamin D and the level of Diabetic Retinopathy

To evaluate whether 25 hydroxy Vitamin D level can be used as a predictor of the severity of Diabetic Retinopathy.

MATERIALS AND METHODS

SUBJECT SELECTION:

40 patients with type 2 diabetes mellitus and diabetic retinopathy were included in this prospective, interventional study which was carried out at the department of vitreo-retina services, Regional Institute of ophthalmology and Government ophthalmic hospital, Madras Medical College, Chennai, over a period of 1 year.

Inclusion criteria:

- Patients must be aged 18 years and above.
- Patients with type 2 Diabetes Mellitus who are diagnosed as Non Proliferative Diabetic Retinopathy or Proliferative Diabetic Retinopathy

Exclusion criteria:

- Patients with type 1 Diabetes
- Patients previously on vitamin D supplementation.
- Use of medications known to influence mineral metabolism including calcitonin, growth hormone, thiazide diuretics, anti-

convulsants such as Carbamazepine, phenytoin and phenobarbitone or excessive doses of vitamin A (> 20,000 units/day).

- History of disorders that are known to affect the metabolism of Vitamin D and major medical illness including renal failure, hepatic dysfunction, and musculoskeletal disorders were excluded
- Serum calcium < 8 or > 11 mg/dL
- Patients on hormone replacement therapy, steroids or testosterone.

PROCEDURE

HISTORY:

All patients were screened with a detailed history including nature & duration of symptoms, duration of exposure to sunlight, diet history and history of tobacco chewing or alcohol consumption. History of treatment with oral hypoglycaemic agents, insulin, vitamin supplements and other drug intake, disorders known to influence Vitamin D levels and previous ophthalmic surgery/laser or other medical treatment history was also taken.

Patients then underwent a complete systemic and ocular examination.

GENERAL EXAMINATION

General vital data like pulse, blood pressure, peripheral pulses were noted. Height (in meters) and weight (in kgs) was recorded and systemic examination of CNS, CVS, RS and abdomen was done.

OCULAR EXAMINATION

- Visual acuity was assessed by Snellen's chart and refractive status was noted
- Anterior segment evaluation with slit lamp biomicroscopy was performed
- Intraocular pressure was measured using Goldmann Applanation tonometer
- Diabetic retinopathy was evaluated by a dilated fundus examination using 90D & indirect ophthalmoscopy
- Fundus photographs were taken for documentation

- All patients will underwent Fundus fluorescein angiography and OCT to assess maculopathy if present
- The levels of retinopathy will then be classified as per the ETDRS.

INVESTIGATIONS

Fasting blood glucose levels (FBS), Post prandial blood glucose levels (PPBS), urine sugar and albumin and serum Calcium were measured. Measurement of serum 25 hydroxy Vitamin D and HbA1C was be done prior to and following 6 months of oral vitamin D supplementation.

Twenty patients received daily oral supplementation of 2000 IU of Vitamin D for a duration of 6 months, constituting the treatment arm, while the other 20 patients served as the control arm.

FOLLOW UP PROCEDURES/ VISITS

Patients were re-examined every month. At each visit visual acuity recording, anterior segment examination by slit lamp biomicroscopy, tonometry and dilated fundus examination was done. Any progression of retinopathy was noted. Fundus photographs were

repeated at each visit. Fundus fluorescein angiography was repeated at 3 months and 6 months. OCT to re-assess maculopathy, was repeated at 3 and 6 months, or earlier in selected patients if deemed necessary. Fasting and post prandial blood glucose levels (FBS and PPBS) and urine sugar and albumin were measured to ensure glycaemia control. Compliance with oral Vitamin D supplementation therapy was ensured by counting the empty medicine strips at each visit and history of Vitamin D toxicity if any was elicited.

ASSESSMENT OF PARAMETERS

- The level and severity of diabetic retinopathy
- Improvement in Best corrected visual acuity (logMAR conversion of Snellen's chart)

BIOCHEMICAL ESTIMATION OF VITAMIN D IN SERUM

In our study, the direct, competitive chemiluminescent immunoassay (CLIA) method was used for quantitative estimation of 25- hydroxy Vitamin D. This is a United States Food and Drug Administration (FDA)–approved immunoassay method. This method was developed in 2002 and measured total 25-hydroxy vitamin D in serum samples. Hepatic metabolism converts Vitamin D3 and D2 to 25-hydroxyvitamin D. Serum or plasma 25-OH vitamin D levels is the best indicator of vitamin D nutritional status.

PERFORMANCE SPECIFICATIONS

The measurement range is from 4 to 150 ng/ml.

SAMPLE REQUIREMENTS

- Human serum samples
- Samples may be collected after an overnight fast, however this is not essential
- Infected and haemolysed samples must be rejected

PREPARATION OF SAMPLES

At least 250 µL of sample is essential for the first test. Frozen samples must be thawed and mixed thoroughly until a homogeneous specimen is obtained.

STORAGE OF SAMPLES

Samples are stored in the frozen state (-20˚C or below) in glass or plastic vials without any additives or preservatives.

REAGENTS

- Anti 25 OH Vitamin D antibody coated magnetic particles
- Conjugate used is an isoluminol derivative, in phosphate buffer with EDTA, preservatives, surfactants and 10% ethanol

PRINCIPLE OF THE TEST

It is a two step incubation procedure. The initial step involves a separation of 25-hydroxy vitamin D from its binding protein, which is followed by making it bind to the specific solid phase antibody. This is followed by the addition of vitamin D-isoluminol tracer after 10 minutes. A wash cycle is then used to remove unbound material

following the second 10 minute incubation period. Chemiluminescent reaction is initiated by adding the starter reagents. The photomultiplier detects the light signal, the strength of which is used to determine the 25-hydroxy vitamin D levels. The light signal, indicated in relative light units bears a negative relation with 25 hydroxy vitamin D levels.

REFERENCE VALUES OF 25 HYDROXY VITAMIN D

Deficiency	:	<20 ng/ml
Insufficiency	:	20 – 30 ng/ml
Sufficiency	:	30 - 100 ng/ml
Toxicity	:	>150 ng/ml

STATISTICAL ANALYSIS

Data analysis was carried out by the Statistical Package For Social Science (SPSS). Quantitative data were presented as means and standard deviation and qualitative data were expressed as numbers or percent.

OBSERVATION AND RESULTS

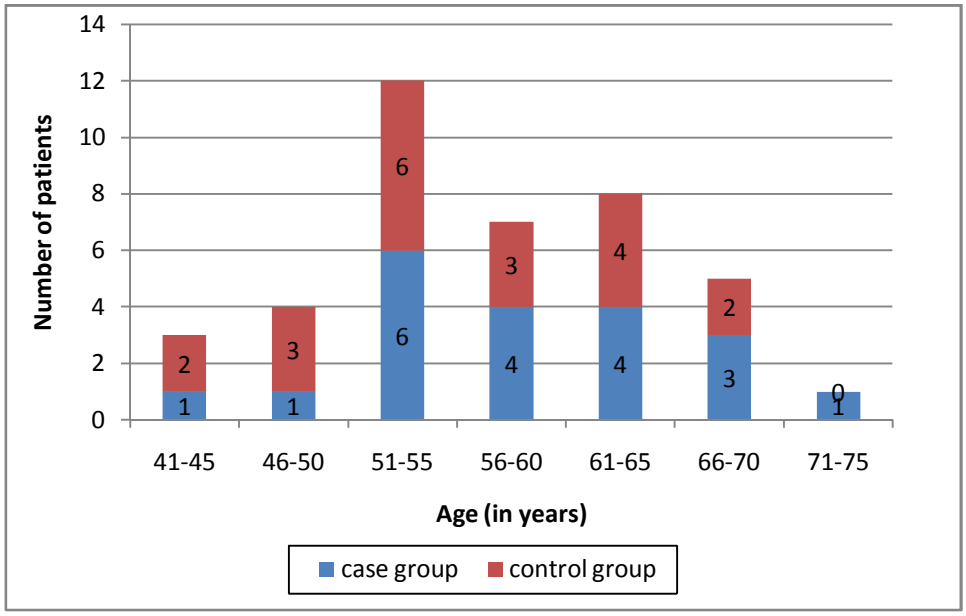
40 patients were enrolled in this study. 20 patients received oral Vitamin D supplementation, serving as the case group and the remaining 20 patients served as the control group.

Age distribution

Table 1. Age distribution of a total of 40 patients in the case and control arm.

Age in years	No. of patients – Case group	No. of patients – control group	Total no. of patients
41-45	1	2	3 (7.5%)
46-50	1	3	4 (10%)
51-55	6	6	12 (30%)
56-60	4	3	7 (17.5%)
61-65	4	4	8 (20%)
66-70	3	2	5 (12.5%)
71-75	1	0	1 (2.5%)

Chart 1. Age distribution



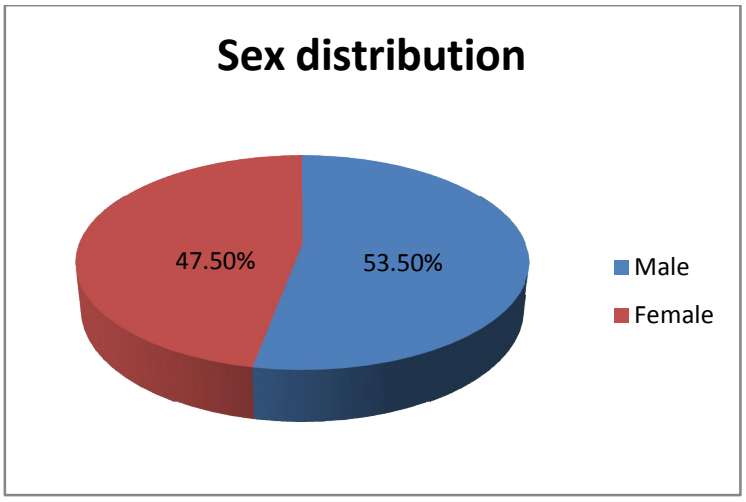
In this study patients between 51-55 years were maximally affected (30%). The oldest patient enrolled was 72 years, while the youngest patient was 43 years. The mean age at presentation was 58.85 ± 7.09 years in the case group and 56.50 ± 7.72 years in the control group.

Sex distribution

Table 2.Distribution of males and females

Sex	No. of patients – case group	No. of patients – control group	Total no. of patients
Male	9	12	21 (53.5%)
Female	11	8	19 (47.5%)

Chart 2. Sex wise distribution



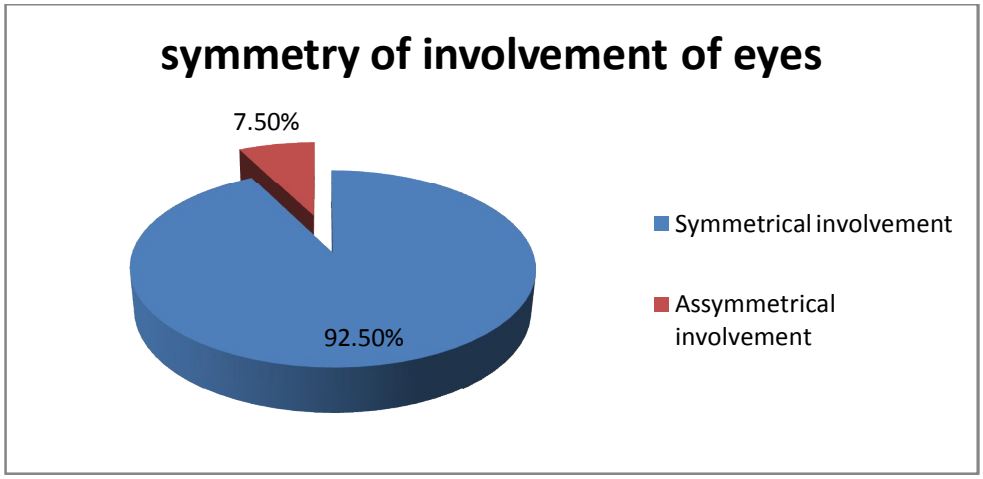
21 (53.5%) males and 19 (47.5%) females were affected with diabetic retinopathy in this study.

Laterality and symmetry

Table 3. Symmetry of involvement of eyes

	No. of patients – case group	No. of patients – control group	Total patients
Symmetrical involvement	18	19	37 (92.5%)
Asymmetrical involvement	2	1	3 (7.5%)

Chart 3. Symmetry of involvement of eyes



All patients enrolled in this study had bilateral disease, however the severity of diabetic retinopathy was asymmetrical in 3 patients (7.5%). The remaining 37 cases (92.50%) had a bilaterally symmetrical presentation of diabetic retinopathy. The age and sex distribution and the symmetry of involvement were not significantly different between the cases and controls ($p>0.05$).

Severity of diabetic retinopathy at presentation

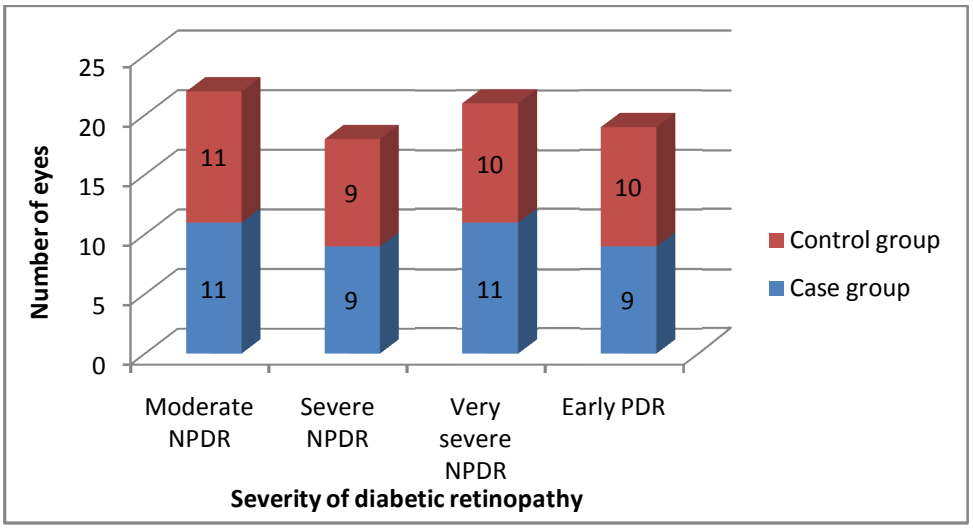
Table 4. Classification of patients based on severity of retinopathy at presentation

Severity of retinopathy	No. of patients – case group	No. of patients – control group	Total patients
Moderate NPDR	5	5	10
Severe NPDR	5	5	10
Very severe NPDR	5	5	10
Early PDR	5	5	10

Table 5.Classification of eyes based on severity of retinopathy at presentation

Severity of retinopathy	No. of eyes in case group	No of eyes in control group	Total no. of eyes
Moderate NPDR	11	11	22 (27.5%)
Severe NPDR	9	9	18 (22.5%)
Very severe NPDR	11	10	21 (26.3%)
Early PDR	9	10	19 (23.8%)

Chart 4.Distribution of eyes based on severity of retinopathy at presentation



In both the case and control group, 5 patients each were diagnosed with moderate NPDR, severe NPDR, very severe NPDR and early PDR. None of the patients were diagnosed with mild NPDR or high-risk PDR at presentation. In patients with asymmetrical disease, the eye with increased severity of retinopathy was used to decide the level of retinopathy to which the patient must be assigned to.

Of the 80 eyes studied, 22 eyes (27.5%) had moderate NPDR, 18 eyes (22.5%) had severe NPDR, 21 eyes (26.3%) had very severe NPDR and 19 eyes (23.8%) had early PDR. The distribution of severity of retinopathy at presentation was not significantly different between the case and control groups by Pearson's Chi Square test (p=0.992).

Duration of Diabetes mellitus

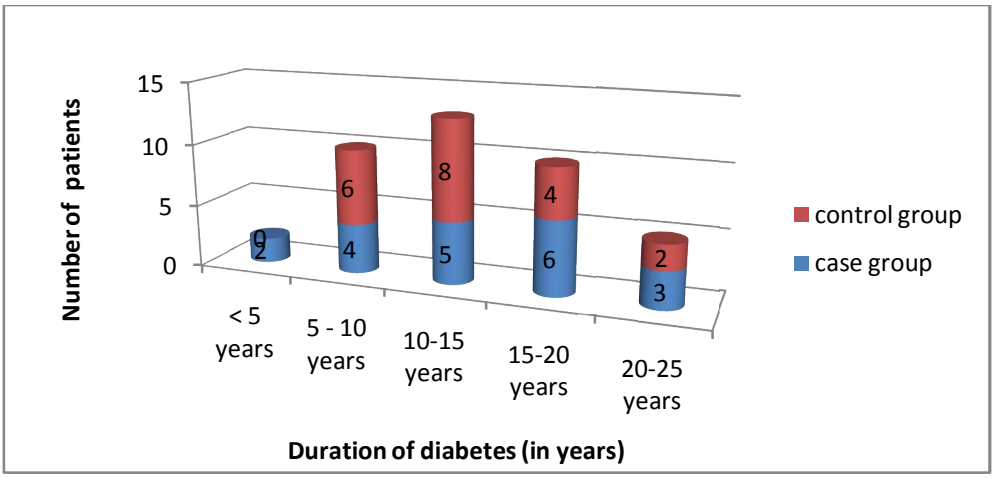
Table 6.duration of diabetes

Duration of diabetes (in years)	No. of patients – case group	No. of patients – control group	Total patients
<5	2	0	2 (5%)
5-10	4	6	10 (25%)
10-15	5	8	13 (32.5%)
15-20	6	4	10 (25%)
20-25	3	2	5 (12.5%)

Table 7.Distribution of the duration of diabetes according to the severity of retinopathy

Severity of retinopathy	Duration of diabetes in cases (in years)	Duration of diabetes in controls (in years)	p-value
Moderate NPDR	9.0 +/- 6.48	8.5 +/- 3.87	0.8963
Severe NPDR	9.0 +/- 3.81	10.50 +/- 4.20	0.592
Very severe NPDR	12.40 +/- 2.51	10.50 +/- 3.32	0.3587
Early PDR	17 +/- 4.47	18.25 +/- 3.86	0.6722
Total	11.6 +/- 5.52	11.74 +/- 5.14	0.976

Chart 5.Duration of diabetes



The duration of diabetes was taken as the interval between the first diagnosis of diabetes mellitus by health personnel and the present study period. A majority of patients (32.5 %) had duration of diabetes between 10-15 years, 25% of patients had duration between 5-10 and 15-20 years, 12.5% had a duration between 20-25 years and the least number of patients had a duration of less than 5 years (5%).

Patients with early PDR had the longest duration, with a mean of 17 years and 18.25 years in the case and control groups respectively. On the other hand, patients with moderate NPDR showed a mean of 9 years and 8.5 years in the case and control groups respectively, being the least.

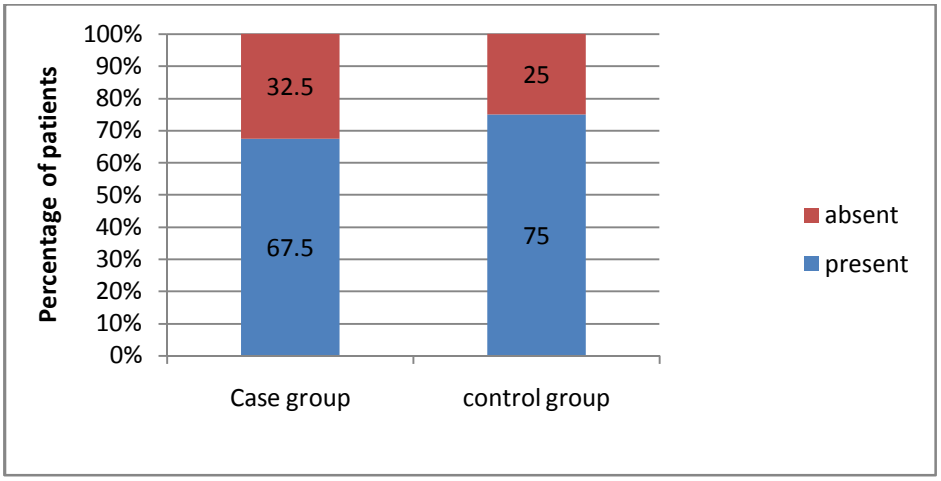
The mean duration of diabetes in the case and control groups was 11.6 years and 11.74 years respectively.

Distribution of maculopathy

Table 8.Distribution of maculopathy

Maculopathy	No. of eyes - cases	No. of eyes – controls	Total eyes
Present	27 (67.5%)	30 (75%)	57 (71.25%)
Absent	13 (32.5%)	10 (25%)	23 (28.75%)

Chart 6.Distribution of maculopathy



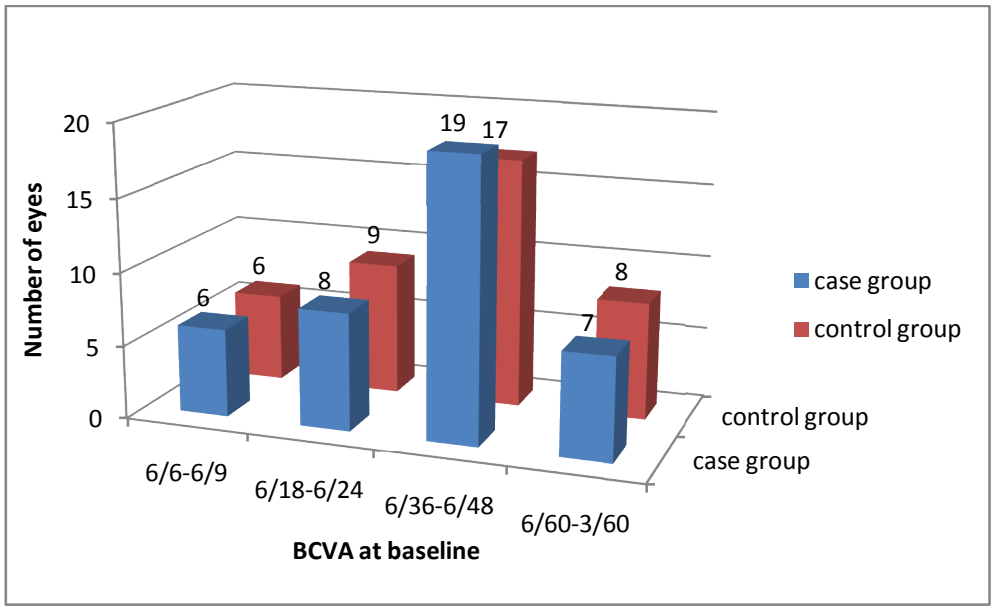
A total of 57 eyes (71.25%) had associated maculopathy with features of diabetic retinopathy. The mean CMT at baseline was 250 ± 59.67 microns in the cases and 252.80 ± 54.65 microns in the controls. The cases and controls did not differ significantly in the duration of diabetes, occurrence of maculopathy or the mean CMT at baseline.

Best Corrected Visual Acuity at baseline

Table 9.BCVA at baseline

Visual Acuity	Case group		Control group	
	No.of eyes	%	No.of eyes	%
3/60 - 6/60	6	15	6	15
6/36 – 6/24	8	20	9	22.5
6/18 – 6/12	19	47.5	17	42.5
6/9 – 6/6	7	17.5	8	20

Chart 7.BCVA at baseline



After logMAR conversion, the mean BCVA was found to be 0.5085 ± 0.33059 in the cases and 0.5250 ± 0.33429 in the controls which was not found to be significantly different, with a two-tailed p-value of 0.828 by unpaired t-test.

Treatment of diabetes mellitus

Table 10.Treatment of diabetes

Treatment of Diabetes	No. of patients-Case group	No. of patients – control group	Total no. of patients
Insulin	10	9	19 (47.5%)
Oral hypoglycaemic agents (OHA)	9	9	18 (45%)
Insulin + OHA	1	2	3 (7.5%)

19 patients (47.5%) were on insulin therapy, 18 patients (45%) were on oral hypoglycaemic agents (OHAs) and 3 patients (7.5%) were on treatment with Insulin and OHAs.

Table 11.Distribution of the treatment of diabetes according to the severity of retinopathy

Treatment of diabetes		**No. of patients- Case group**	**No. of patients – control group**	**Total no. of patients**
Moderate NPDR	OHA	5	5	10
Severe NPDR	OHA	4	4	8
	Insulin	1	1	2
Very severe NPDR	Insulin	4	4	8
	Insulin + OHA	1	1	2
Early PDR	Insulin	5	4	9
	Insulin + OHA	0	1	1

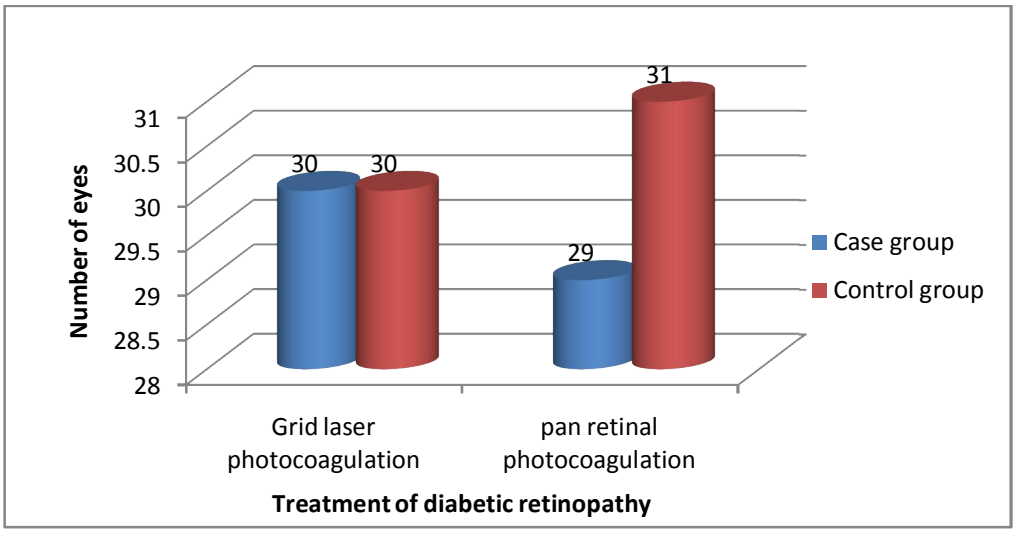
While a majority of patients with early PDR, very severe and severe NPDR were receiving insulin therapy, all patients diagnosed with moderate NPDR were receiving treatment with OHAs alone.

Treatment of retinopathy

Table 12.Treatment of retinopathy

Treatment of retinopathy	No. of eyes – cases	No. of eyes - controls
Grid laser photocoagulation	30	30
Pan-retinal photocoagulation	29	31

Chart 8.Distribution of retinopathy



Patients in the case as well as control group continued to receive laser treatment in the form of pan-retinal photocoagulation or grid photocoagulation during the course of the study. The number of eyes receiving grid laser photocoagulation was 30 patients each in the case and control groups, while those receiving pan-retinal photocoagulation were 29 and 31 patients respectively in the case and control groups.

Associated systemic diseases

Table 13.distribution of associated hypertension

	No. of patients with hypertension	**Percentage**
Case group	6	15%
Control group	5	12.5%

15% of cases and 12.5% of controls had associated systemic hypertension. None of the patients enrolled in the study had other systemic diseases. The groups did not differ significantly in the treatment of diabetes mellitus or retinopathy or associated systemic diseases (p-value > 0.05).

HbA1c levels at baseline

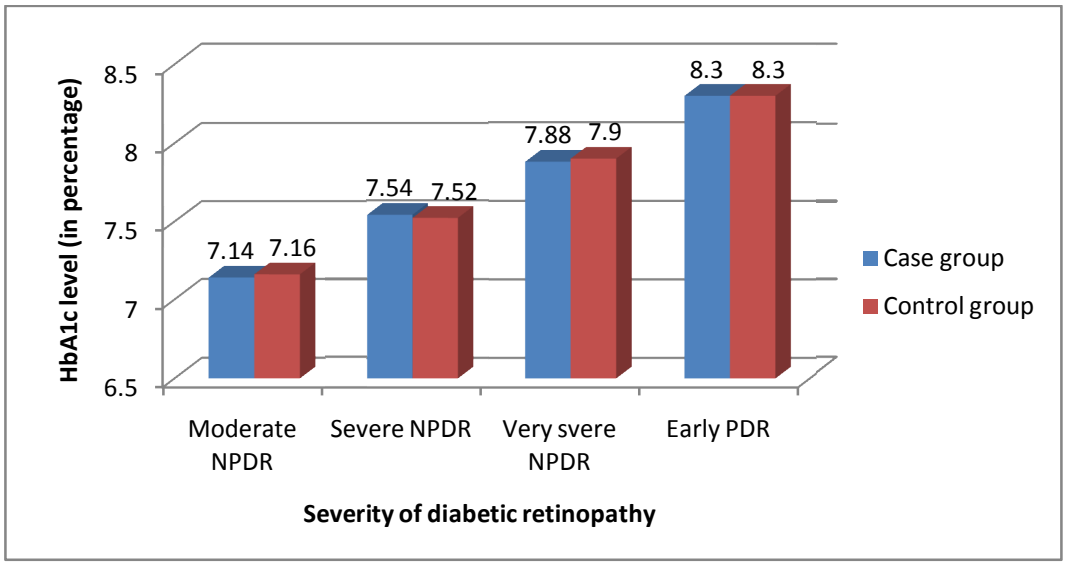
Table 14.Distribution of HbA1c levels according to the severity of retinopathy

Severity of retinopathy	Mean (in %)	Standard deviation	Mean (in %)	Standard deviation	P value
Moderate NPDR	7.140	0.270	7.160	0.288	0.9126
Severe NPDR	7.540	0.055	7.520	0.084	0.6666
Very severe NPDR	7.880	0.084	7.9	0.082	0.7294
Early PDR	8.3	0.1	8.3	0.115	1.0
Total	7.715	0.460	7.715	0.459	1.0

Table 15.Distribution of HbA1c levels

Serum HbA1c levels	No. of patients				
	Moderate NPDR	Severe NPDR	Very severe NPDR	Early PDR	Total patients
6.0%-7.0%	4	0	0	0	4 (10%)
7.0%-8.0%	6	10	8	0	24 (60%)
8.0%-10%	0	0	2	10	12 (30%)

Chart 9.Distribution of HbA1c levels according to severity of retinopathy



The mean serum HbA1c levels were 7.715 ± 0.460% in cases and 7.715 ± 0.459% in controls, which was not found to be significantly different (two-tailed p value=1.0 by unpaired t-test). The levels of serum HbA1c were highest in patients with early PDR in both cases and controls and progressively decreased with increasing severity of retinopathy, being lowest in patients with moderate NPDR.

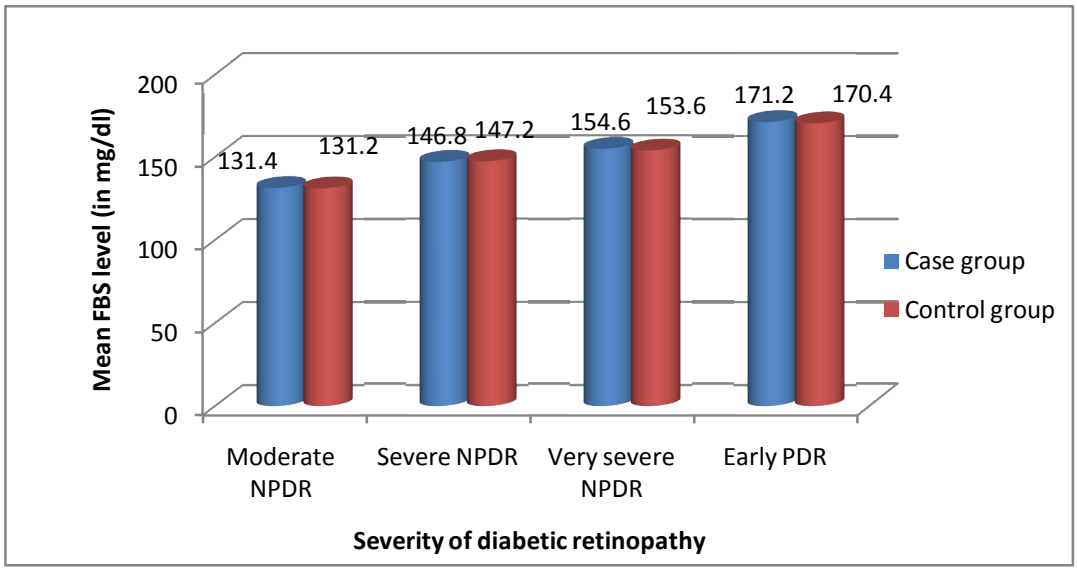
Of the 40 patients enrolled in the study, a majority (60%) of the patients demonstrated fair gylcaemic control (serum HbA1c = 7.0%-8.0%), 30% of the patients showed unsatisfactory control (serum HbA1c = 8.0%-10.0%) and the remain 10% of patients demonstrated good glycaemic control (serum HbA1c = 6.0%-7.0%)

Serum FBS levels

Table 16.FBS Levels in diabetic retinopathy

Severity of retinopathy	FBS levels				
	Mean (in mg/dl)	Standard deviation	Mean (in mg/dl)	Standard deviation	P value
Moderate NPDR	131.4	8.96	131.25	11.44	0.9830
Severe NPDR	146.8	6.42	147.2	10.43	0.9436
Very severe NPDR	154.6	4.04	153.6	3.71	0.6943
Early PDR	171.2	7.09	170.4	8.11	0.8722
Total	151.0	16.0	150.8	16.14	0.9688

Chart10.Baseline serum FBS levels in diabetic retinopathy



The mean serum fasting blood sugar levels were 151 ± 16 mg/dl and 150 ± 16.14 mg/dl in the cases and control groups respectively, which did not differ significantly (two-tailed p value=0.9688 by unpaired t-test). The levels of serum FBS were highest in patients with early PDR in both cases and controls and progressively decreased, being lowest in patients with moderate NPDR. The levels of both HbA1c and FBS did not differ significantly between cases and controls having moderate, severe, very severe NPDR and early PDR ($p > 0.05$).

Serum 25 hydroxy Vitamin D levels at baseline

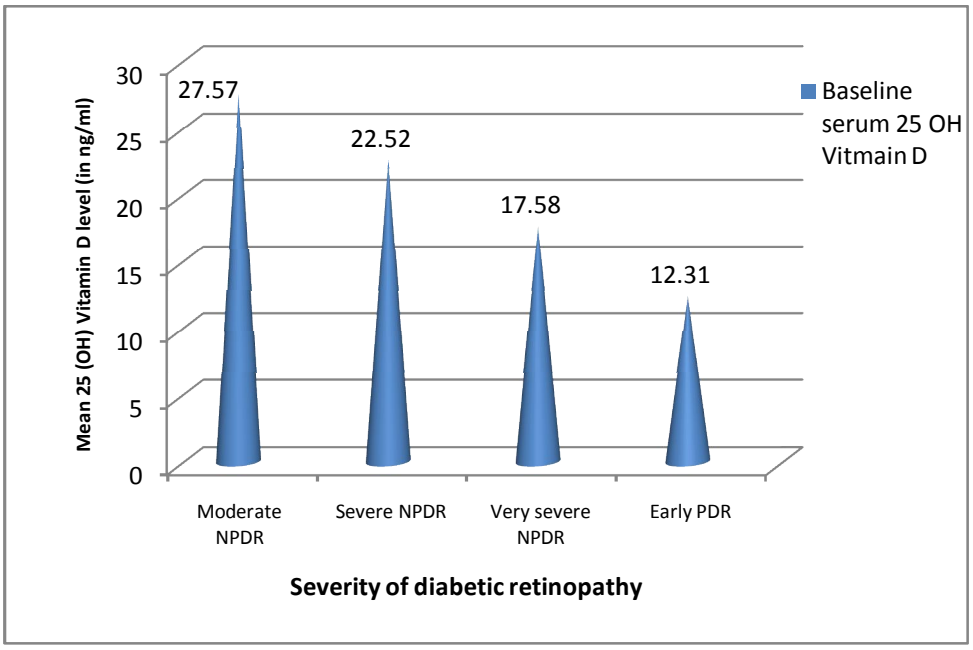
Table 17.Distribution of baseline serum 25 hydroxy vitamin D levels

Serum 25 hydroxy Vitamin D levels (ng/ml)	No. of patients				
	Moderate NPDR	Severe NPDR	Very severe NPDR	Early PDR	Total patients
<20	8	8	9	10	35 (87.5%)
20-30	1	1	1	0	3 (7.5%)
30-100	1	1	0	0	2 (5%)
>100	0	0	0	0	0

Table 18. Distribution of baseline serum 25 hydroxy vitamin D levels according to severity of retinopathy

Severity of retinopathy	Mean Vitamin D levels (ng/ml)	Standard deviation	Minimum Vitamin D levels (in ng/ml)	Maximum vitamin D level (in ng/ml)
Moderate NPDR	27.573	6.0525	18.8	42.5
Severe NPDR	22.518	3.6956	18.3	32.3
Very severe NPDR	17.557	1.7105	15.8	21.1
Early PDR	12.131	2.7112	9.1	17.7
Total	19.945	6.9074	9.1	42.5

Chart 11.Mean 25 hydroxy Vitamin D levels at baseline according to severity of retinopathy



A total of 35 patients (87.5%) enrolled in the study were found to have Vitamin D deficiency (serum 25 hydroxy vitamin D: < 20 ng/ml), 3 patients (7.5%) were found to have Vitamin D insufficiency (serum 25 hydroxy vitamin D : 20-30 ng/ml) and 2 patients (5%) were found to have normal Vitamin D levels.

By One way ANOVA with Post Hoc tests and Tukey HSD, a highly significant p value of less than 0.001 was obtained, indicating that the levels of serum 25 hydroxy Vitamin D measured in the 40 patients at baseline were significantly lowered as the severity of

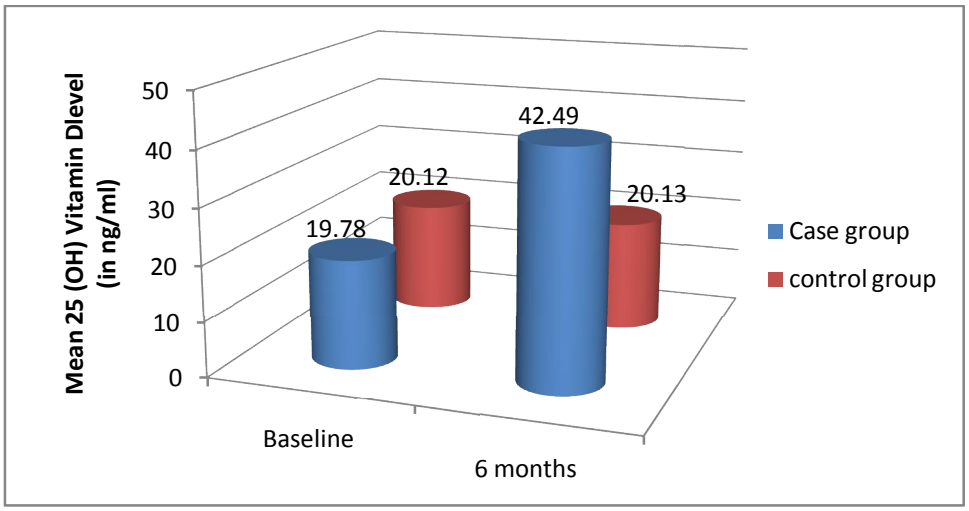
retinopathy increased from moderate NPDR to early PDR. The patients with moderate NPDR had a mean value of serum 25 hydroxy vitamin D of 27.573 ng/ml, which decreased to 22.518 ng/ml and 17.557 ng/ml in patients with severe and very severe NPDR respectively and patients with early PDR had the lowest value of serum 25 hydroxy vitamin D of 19.945 ng/ml.

Pearson correlation test, showed a two-tailed significance of -0.841, indicating a strongly negative correlation between serum 25 hydroxy Vitamin D levels with the severity of diabetic retinopathy as documented at baseline in all 40 patients. Hence as the serum levels of 25 hydroxy Vitamin D decreased, the severity of diabetic retinopathy was found to increase.

The mean serum 25 hydroxy Vitamin D levels were 19.775 ± 5.663 ng/ml and 20.115 ± 8.101 ng/ml in the case and control groups respectively at presentation and did not differ significantly between the two groups, having a two-tailed p value of 0.8793 by the unpaired t-test.

Serum 25 hydroxy Vitamin D levels at 6 months

Chart12.Serum 25 hydroxy Vitamin D at baseline and 6 months



The mean serum 25 hydroxy Vitamin D levels were 42.4930 ± 9.9332 ng/ml and 20.1305 ± 1.7346 ng/ml in the case and control group respectively at the end of 6 months, which was significantly higher in the case group following 6 months of oral supplementation than the control group. A two-tailed p value of 0.8793 by the unpaired t-test was obtained.

The mean serum 25 hydroxy Vitamin D levels were 20.1150 ± 8.1012 ng/ml and 20.1305 ± 7.7575 ng/ml in the control group at baseline and at 6 months respectively, showing a no significant difference with a two-tailed p value of 0.9585 by the paired t-test.

Table 19.Distribution of serum 25 hydroxy Vitamin D levels at 6 months in cases

Serum 25 hydroxy Vitamin D3 levels (ng/ml) in cases at 6 months	No. of patients	percentage
<20	0	0
20-30	4	10%
30-100	16	80%
>100	0	0

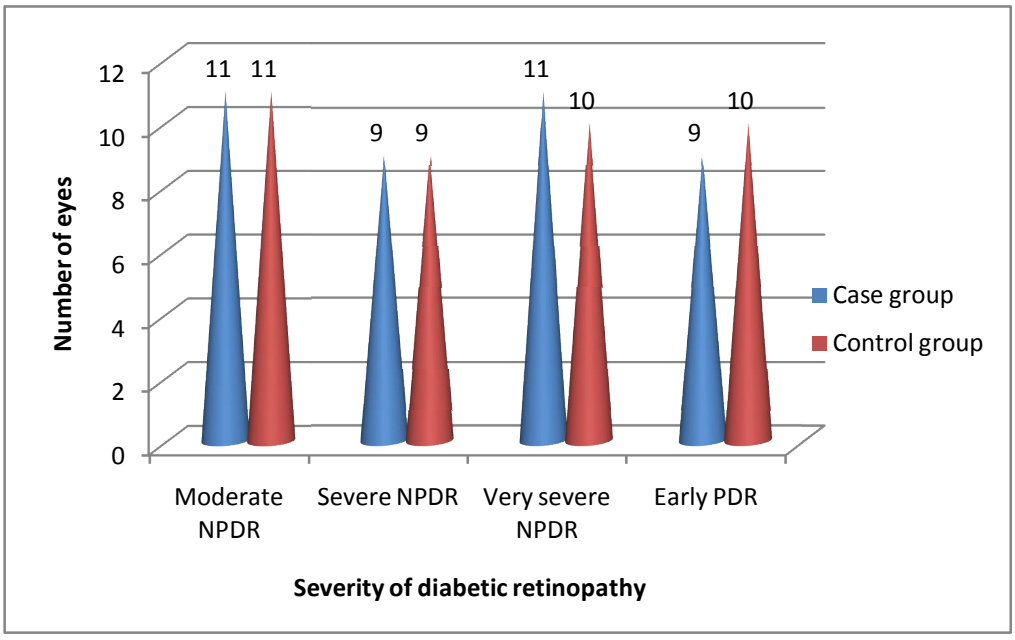
Following 6 months of oral supplementation of Vitamin D, 80% cases attained normal serum 25 hydroxy vitamin D levels (30-100 ng/ml), 10% patients remained insufficient (20-30 ng/ml) and none of the patients remained deficient (<20 ng/ml). None of the cases attained toxic serum levels of 25 hydroxy vitamin D (>100 ng/mg).

Severity of retinopathy – at 1 month follow up

Table 20.Severity distribution of retinopathy at 1 month

Severity of retinopathy at 1 month	No. of eyes in case group	Percentage in case group	No of eyes in control group	Percentage in control group
Moderate NPDR	11	27.5%	11	27.5%
Severe NPDR	9	22.5%	9	22.5%
Very severe NPDR	11	27.5%	10	25%
Early PDR	9	22.5%	10	25%

Chart 13.Severity distribution at 1 month



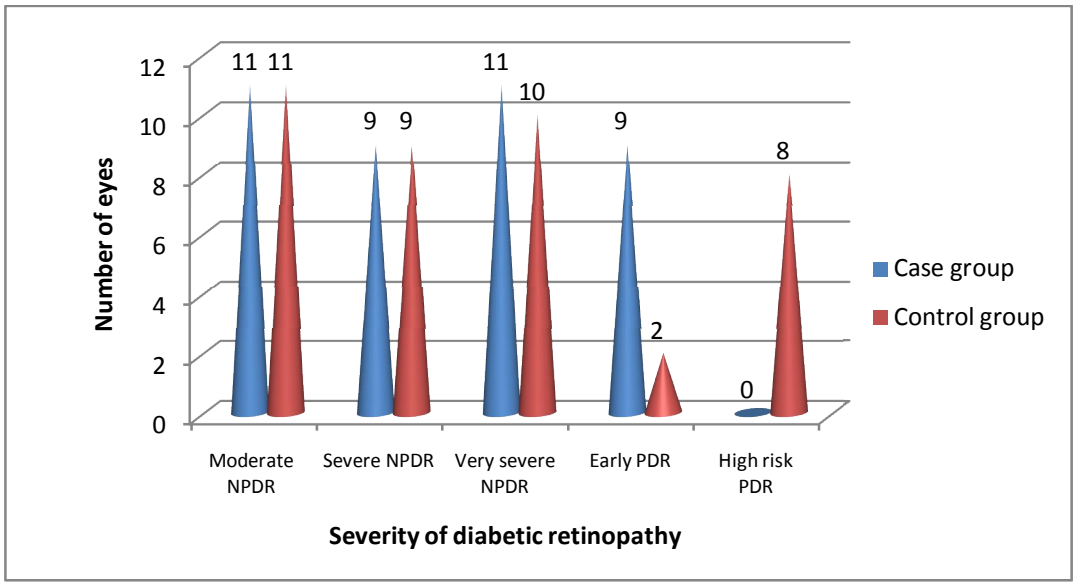
At 1 month follow up, 27.5% of eyes in both the case and control groups were found to have moderate NPDR and 22.5% eyes in both groups had severe NPDR. 27.5% of eyes among the cases had very severe NPDR, compared to 25% of controls and 22.5% of eyes in the case group had early PDR compared to 25% of eyes of controls. The severity of diabetic retinopathy noted at 1 month follow up did not differ significantly between the cases and controls (2-sided p=0.992 according to Pearson Chi-Square test).

Severity of retinopathy – at 3 months follow-up

Table 21.Severity distribution of retinopathy at 3 months

Severity of retinopathy at 3 month	No. of eyes in case group	Percentage in case group	No of eyes in control group	Percentage in control group
Moderate NPDR	11	27.5%	11	27.5%
Severe NPDR	9	22.5%	9	22.5%
Very severe NPDR	11	27.5%	10	25%
Early PDR	9	22.5%	2	5%
High-risk PDR	0	0	8	20%

Chart 14.Severity distribution at 3 months



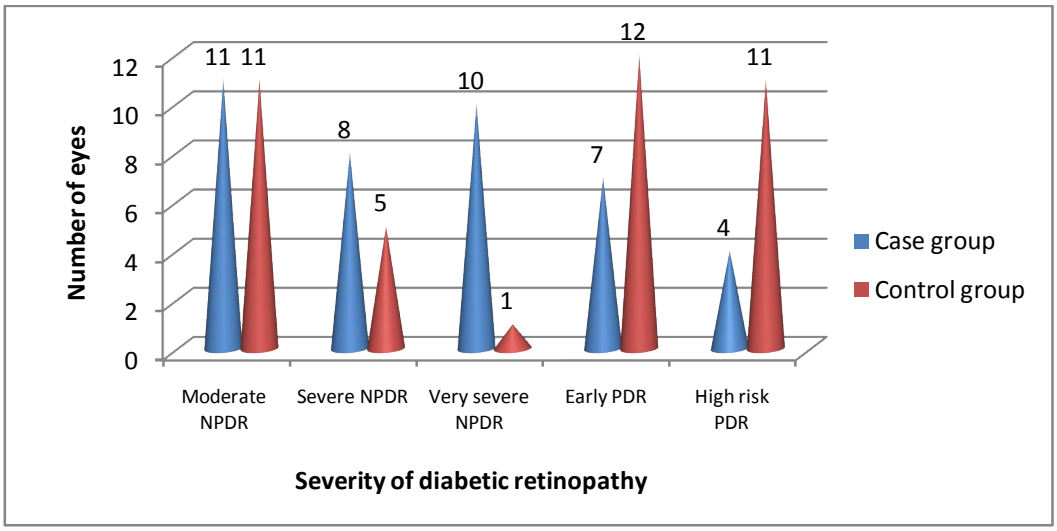
At 3 months, there was no progression noted in the patients found to have moderate, severe and very severe NPDR in both groups, with 27.5% of the eyes of cases and controls having moderate NPDR and 22.5% of the eyes patients in both groups having severe NPDR. 27.5% of cases had very severe NPDR, compared to 25% of controls and 22.5% of cases had early PDR compared to 25% of controls. 27.5% and 25% of the eyes of cases and controls respectively were found to have very severe NPDR. 22.5% eyes with early PDR in the case group did not progress at 3 months. However in the control group 8 eyes (20%) with early PDR at 1 month progressed to high risk PDR. This progression from early PDR to high-risk PDR in the control group, compared to no such progression among the cases was found to be statistically significant (2-sided p=0.014 according to Pearson Chi-Square test).

Severity of retinopathy - at 6 months

Table 22. Severity distribution of retinopathy at 6 months

Severity of retinopathy at 6 month	No. of eyes in case group	Percentage in case group	No of eyes in control group	Percentage in control group
Moderate NPDR	11	27.5%	11	27.5%
Severe NPDR	8	20%	5	12.5%
Very severe NPDR	10	25%	1	2.5%
Early PDR	7	17.5%	12	30%
High-risk PDR	4	10%	11	27.5%

Chart 15. Severity distribution at 6 months



At 6 months, none of the eyes with moderate NPDR progressed in both groups, with 27.5% of the eyes of cases and controls having moderate NPDR. Severe NPDR was found in 20% and 12.5 % of eyes of cases and controls respectively. Very severe NPDR was found in 25% of eyes of cases and 2.5% of eyes of controls. 17.5% and 30% of eyes of the cases and controls were found to have early PDR. High risk PDR had developed in 10% of eyes of cases and 27.5% of eyes of controls. Hence at 6 months majority of eyes of cases showed moderate NPDR while majority of eyes of controls showed early PDR.

Hence at 6 months, eyes with severe, very severe NPDR and early PDR were found to have progressed in both groups, however the progression was significantly greater in cases then in controls (2-sided p=0.013 according to Pearson Chi-Square test).

Maculopathy at 6months

Table 23.Distribution of maculopathy at 6 months

maculopathy	No. of eyes - cases	No. of eyes - controls	Total eyes
Present	9	10	19
Absent	31	30	61

The mean CMT was 194.67 ± 50.67 microns in cases and 197.08 ± 34.40 microns in controls which did not differ significantly between the two groups at the end of 6 months ($p>0.05$).

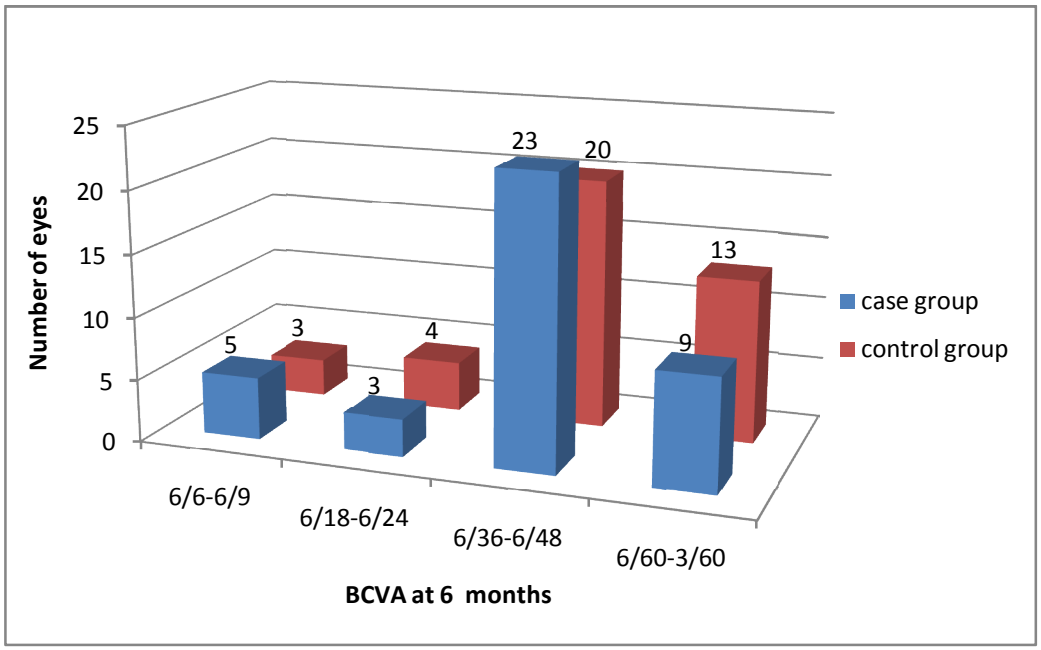
The mean CMT was found to decrease significantly in both the cases and controls at the end of 6 months with a two-tailed p value = 0.0001 by paired t-test in both groups.

BCVA at 6 month

Table24.BCVA at 6 months

Visual Acuity	Case group		Control group	
	No.of eyes	%	No.of eyes	%
3/60 - 6/60	5	12.5	3	7.5
6/36 – 6/24	3	7.5	4	10
6/18 – 6/12	23	57.5	20	50
6/9 – 6/6	9	22.5	13	32.5

Chart 16.BCVA at 6 months



After logMAR conversion, the mean best corrected visual acuity was found to be 0.4265 ± 0.3311 in the cases and 0.3750 ± 0.2507 in the controls which was not found to be significantly different, with a two-tailed p-value of 0.435 by unpaired t-test.

After logMAR conversion at 6 months, the mean best corrected visual acuity improved significantly from 0.5085 ± 0.3360 at baseline to 0.4265 ± 0.3311 at 6 months in the 40 eyes enrolled in the case group (two-tailed p value=0.0324 by paired t-test). A significant improvement was also found in the 40 eyes of controls from a mean BCVA of 0.5250 ± 0.3443 at baseline to 0.3750 ± 0.2507 at 6 months (two-tailed p value=0.0008 by paired t-test).

DISCUSSION

Diabetic retinopathy remains one of the leading causes of visual loss in the working-age people. After duration of diabetes exceeding 15 years, it affects three out of four diabetic patients.

In our study 75% of patients belonged to the 6^{th} and 7^{th} decades of life. This may be due to the fact that prevalence of systemic diseases such as diabetes and hence its resulting microvascular complications, increases with increasing age. The youngest patient enrolled was 43 years and the oldest was 72 years. Previous studies revealed a similar age distribution. (Daniel M.Taylor et al. Survey ophthalmology).[59]

In our study, 53.5% males and 47.5% females were affected with diabetic retinopathy. Although an increased percentage of males were found to be affected, no significant male preponderance was observed. This finding was consistent with previous studies.[14.15]

All patients enrolled in this study had bilateral disease, however the severity of diabetic retinopathy was asymmetrical in 7.5% of patients.

Among the 80 eyes studies, 27.5% had moderate NPDR, 22.5% had severe NPDR, 26.3% had very severe NPDR and 23.8% had early PDR. 71.5% eyes had maculopathy associated with retinopathy. In the Oman study, 60% of the eyes had NPDR, 18% had PDR and 24% of eyes had associated maculopathy. This increased incidence in our study, could be attributed to the late presentation of diabetic patients for ophthalmological screening due to decreased awareness.[60]

We found that a majority of 32.5 % of patients with retinopathy had duration of diabetes between 10-15 years. As expected, in patients with a greater duration of diabetes, an increased severity of retinopathy was observed. Patients with early PDR had the longest duration, with almost all cases having duration of more than 10 years. On the other hand, patients with moderate NPDR showed duration of less than 10 years. These findings were consistent with the Oman study and the study by Jernald B Alguere.[60,61]

In our study, baseline mean serum HbA1c levels were 7.715 +/- 0.460% in cases and 7.715 +/- 0.459% in controls. The baseline mean serum fasting blood sugar levels were 151 +/- 16 mg/dl and 150 +/- 16.14 mg/dl in the cases and control groups respectively. The levels of

serum HbA1c and fasting blood glucose were highest in patients with early PDR progressively decreased, being lowest in patients with moderate NPDR, findings that were supported by the DCCT study and the study by Kroc Collaborative Study Group.[62-66]

87.5% of patients enrolled in the study were found to have Vitamin D deficiency (serum 25 hydroxy vitamin D: < 20 ng/ml), 7.5% were found to have Vitamin D insufficiency (serum 25 hydroxy vitamin D : 20-30 ng/ml) and 2 patients 5% were found to have normal Vitamin D levels.

The levels of serum 25 hydroxy Vitamin D measured in the 40 patients at baseline were significantly lower as the severity of retinopathy increased from moderate NPDR to early PDR. The patients with moderate NPDR had a mean value of serum 25 hydroxy vitamin D of 27.573 ng/ml, which decreased to 22.518 ng/ml and 17.557 ng/ml in patients with severe and very severe NPDR respectively and patients with early PDR had the lowest value of 19.945 ng/ml. Hence our study demonstrated an inverse relationship between the severity of diabetic retinopathy and serum 25 hydroxy Vitamin D levels, findings which were supported by several other studies.

A strongly negative correlation between serum 25 hydroxy Vitamin D levels and severity of diabetic retinopathy was documented, suggesting that neovascularization in the retina may involve a decrease in serum 25(OH) D concentrations in patients with diabetic retinopathy. Hence, low serum levels of 25 (OH) D may have an association with uncontrolled angiogenesis and the progression of DR as demonstrated by Aksoy H. et. Al.[67]

A cross-sectional study of type 2 diabetic patients conducted by Suzuki A et. Al. showed a significant association between the existence of proliferative retinopathy and a decrease in 25(OH) D.[68] Investigators found a decrease in 25(OH) D according to the number of microvascular complications present. These findings were also supported by a study conducted by Joergensen C et. Al.[69] A clinic-based, cross-sectional study by Paynes et. Al. to assess the relationship between vitamin D status and diabetic retinopathy concluded that diabetic subjects, especially those with PDR, have lowered 25(OH) D levels than those without diabetes.[70] Our findings were also in agreement with the study done by Hulya et al. who demonstrated an inverse relationship between the severity of the retinopathy and retinal neovascularisation with serum 1,25(OH) 2 D3 concentrations, levels

being the lowest in PDR and the highest in diabetic patients without retinopathy.

In order to evaluate whether Vitamin D supplementation delayed the progression of diabetic retinopathy, 40 eyes each in the case and control group were followed up, on a monthly basis for 6 months and the severity of retinopathy was documented. The case and control groups did not differ significantly in the age, sex distribution, duration of diabetes, associated systemic diseases such as hypertension, treatment modalities of diabetes and retinopathy, severity distribution of retinopathy, associated maculopathy and serum HbA1c, fasting blood glucose and 25 hydroxy vitamin D.

The mean serum 25 hydroxy Vitamin D level of 42.4930 ng/ml was attained in the cases following 6 months of oral supplementation, suggesting that this dose used was adequate for normalisation of serum vitamin D levels according to our study. The mean levels remained deficient (20.1305 ng/ml) in the controls at the end of 6 months.

There was a wide variation in the duration, dosage and mode of supplementation used in previous studies. The present “no observed

adverse effect limit" of 2000 IU daily has been found to be too low at least by 5-fold.[71]

In human beings, serum 25 hydroxy vitamin D has been found to have a half life of about 1 month. A minimum of 4 half lives are required before a drug attains an equilibrium concentration. However the concentrations of serum 25 hydroxy vitamin D have been found to change depending on its synthesis and clearance. Hence it has been suggested that it attains a stable serum concentration much earlier. Moreover Davie MW et. Al. demonstrated that 25 hydroxy vitamin D attains a plateau level by 1 month of supplementation. Hence an oral dose of 2000 IU daily was used for 6 months supplementation in our study.

At 1 month follow up, none of the 80 eyes showed progression. At 3 months, there was no progression noted in the patients with moderate, severe and very severe NPDR in both groups. However a significantly increased progression from early PDR to high-risk PDR was found in the control group. While none of the eyes with early PDR in the case group were found to have progressed, 8 eyes (20%) with early PDR in the control group at 1 month had progressed to high risk PDR.

At 6 months, none of the eyes with moderate NPDR progressed in both groups, however eyes with severe, very severe NPDR and early PDR were found to have progressed in both groups, this progression being significantly greater in controls then cases. Severe NPDR was found in 20% and 12.5 % of eyes of cases and controls respectively. Very severe NPDR was found in 25% of eyes of cases and 2.5% of eyes of controls. 17.5% and 30% of eyes of the cases and controls were found to have early PDR. High risk PDR had developed in 10% of eyes of cases and 27.5% of eyes of controls.

The BCVA and maculopathy was found to improve significantly from baseline in both cases and controls, however no difference in BCVA or maculopathy was observed between the two groups at 6 months. This suggests that no additional improvement in BCVA or maculopathy was obtained with vitamin D supplementation, over and above the recommended treatment for diabetic retinopathy.

None of the cases developed any complications relating to Vitamin D toxicity at this dosage recommendation.

SUMMARY

40 patients with type 2 diabetes mellitus and diabetic retinopathy were included in this prospective, interventional study which was carried out at the department of vitreo-retina services, Regional Institute of ophthalmology and Government ophthalmic hospital, Madras Medical College, Chennai, over a period of 1 year.

The aim was to evaluate the effect of supplementation of Vitamin D in delaying the progression of diabetic retinopathy, to study the association of serum 25 hydroxy Vitamin D with the level of Diabetic Retinopathy, and its use as a predictor of the severity of Diabetic Retinopathy.

20 patients received daily oral supplementation of 2000 IU of Vitamin D for a period of 6 months constituting the case arm, while the other 20 patients served as the control arm.

75% of patients enrolled in the study belonged to the 6^{th} and 7^{th} decades of life. 53.5% males and 47.5% females were affected with diabetic retinopathy in this study.

Among the 80 eyes studies, 27.5% had moderate NPDR, 22.5% had severe NPDR, 26.3% had very severe NPDR and 23.8% had early PDR. 71.5% eyes had maculopathy associated with retinopathy.

Our study demonstrated an inverse relationship between the severity of diabetic retinopathy and serum 25 hydroxy Vitamin D levels at baseline. Patients with moderate NPDR had a mean serum 25 hydroxy vitamin D of 27.573 ng/ml, which decreased to 22.518 ng/ml and 17.557 ng/ml in patients with severe and very severe NPDR respectively and patients with early PDR had the lowest value of 19.945 ng/ml.

The mean serum 25 hydroxy Vitamin D level of 42.4930 ng/ml was attained in the cases following 6 months of oral supplementation.

At 1 month follow up, none of the 80 eyes showed progression. At 3 months, there was no progression noted in the patients with moderate, severe and very severe NPDR in both groups. 8 eyes (20%) with early PDR in the control group at 1 month had progressed to high risk PDR. No such progression was noted in cases with early PDR.

At 6 months, none of the eyes with moderate NPDR progressed in both groups, however eyes with severe, very severe NPDR and early PDR were found to have progressed in both groups, this progression being significantly greater in controls then cases.

Severe NPDR was found in 20% and 12.5 % of eyes of cases and controls respectively. Very severe NPDR was found in 25% of eyes of cases and 2.5% of eyes of controls. 17.5% and 30% of eyes of the cases and controls were found to have early PDR. High risk PDR had developed in 10% of eyes of cases and 27.5% of eyes of controls.

The BCVA and maculopathy was found to improve significantly from baseline in both cases and controls, however no difference in BCVA or maculopathy between the two groups at 6 months.

None of the cases developed any complications relating to Vitamin D toxicity.

LIMITATIONS OF THE STUDY

Limitations of our study were the small sample size and short follow up period of 6 months. Larger study group is necessary to quantify the actual magnitude of benefit of vitamin D supplementation in diabetic retinopathy. Longer follow up is necessary to document whether the beneficial effect of such supplementation is seen in mild and moderate NPDR as well.

CONCLUSION

From the results of our study we can conclude that oral supplementation of Vitamin D in addition to the recommended treatment with photocoagulation, delays the progression of severe, very severe and early PDR.

This beneficial effect was observed with an oral dose of 2000 IU daily for 6 months. No toxicity of Vitamin D was noted at this dosage.

An inverse relation between the level of Serum 25 hydroxy vitamin D concentrations and the severity of diabetic retinopathy was noted.

Our study demonstrated that low levels of vitamin D may be a risk marker of development or progression of diabetic retinopathy. It may be advisable to conduct ophthalmologic examination at increased frequency in diabetics whose serum 25 hydroxy vitamin D concentrations are diminished. Serum 25 hydroxy vitamin D concentrations could become a useful biochemical marker to predict the severity of DR in patients with diabetes mellitus in the future.

No additional improvement in BCVA or maculopathy was obtained with vitamin D supplementation, over and above the recommended treatment for diabetic retinopathy.

Further studies with a longer follow-up period and larger sample size are warranted to assess the association between serum 25 hydroxy vitamin D levels and diabetic retinopathy and the efficacy and safety of vitamin D supplementation in delaying the progression of diabetic retinopathy.

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PROFORMA

TITLE: "A clinical study of Vitamin D supplementation in Diabetic Retinopathy patients with type 2 Diabetes Mellitus"

NAME:

AGE:

(Identify certificate having DOB/ Educational certificate/ Marital status and family history/ Appearance)

SEX:

DATE:

HOSPITAL No.:

OCCUPATION:

ADDRESS:

CONTACT No.:

UNIT:

CLINICAL HISTORY

History of presenting complaints:

H/O defective vision in one/both eyes

H/O diminution of vision in one/both eyes:

- Gradual/sudden
- Mild/moderate/severe
- Duration

H/O associated eye pain, redness, watering, photophobia, metamorphopsia, coloured halos, positive scotoma

H/O wearing glasses in the past (if so – refractive status)

H/O any previous ocular surgeries

H/O any ocular trauma

H/O drug intake

H/O any multivitamin intake

PAST HISTORY:

H/O diabetes mellitus

Type of diabetes: IDDM/NIDDM

- Duration
- Age of onset of diabetes
- Treatment for diabetes – OHA/insulin/both
- Whether diabetes is under control or not
- Severity of diabetic retinopathy
- Treatment for diabetic retinopathy: PRP/Grid/Focal (no. Of sittings)

H/O any other systemic diseases: TB/asthma/Ischemic heart disease/nephropathy/Hypertension (duration, treatment taken, whether it is under control or not)

PERSONAL HISTORY:

Diet

H/O tobacco intake (smoking beedi/cigarette)

H/O alcohol intake

Amount of outdoor exposure to sunlight

Education

Marital status

GENERAL EXAMINATION

Height (in meters)

Weight (in kgs)

BMI

Nutritional status

Blood pressure

Skin and Musculoskeletal system

CVS

CNS

RS

Abdomen

OCULAR EXAMINATION

RE LE

Visual acuity

UCVA/BCVA/VA with PH

EOM

Lids

Conjunctiva

Cornea

Iris

Pupil

Anterior chamber

Lens

Intraocular pressure

Duct

Refractive status

Fundus: Distant direct, Direct, IDO and 90D-

- Media
- Disc
- Vessels:
    - AV ratio
    - Venous changes-dilatation, beading, looping. segmentation
    - Arteriolar narrowing, obliteration

- Retinal background – microaneurysms, dot and blot or flame shaped hemorrhages, hard exudates, soft exudates, retinal edema, IRMA
- Macula – hemorrhage, edema, exudates
- NVD/NVE
- Tractional retinal detachment

Fundus photo

FFA-

- leakage
- Areas of capillary non perfusion
- Neovascularisation
- IRMA

OCT- macular thickness

Diagnosis

Colour vision

Fields

IVESTIGATION:

For Serum 25 hydroxy Vitamin D levels:

- Date and time of sample collection
- Result

- Methodology of 25 hydroxy vitamin D estimation
- Follow up visits
- Compliance: by counting empty drug strips
- Evidence of Vitamin D toxicity

FBS/PPBS

Urine albumin and sugar

Serum cholesterol

Serum Calcium

Serum HbA1c

FOLLOW UP VISITS

- BCVA/IOP/Anterior segment/Fundus/Fundus photography
    - 1month
    - 2 months
- 3 months- BCVA/IOP/Anterior segment/Fundus/Fundus photography/FFA/OCT
- BCVA/IOP/Anterior segment/Fundus/Fundus photography
    - 4 months
    - 5 months
- 6 months- BCVA/IOP/Anterior segment/Fundus/Fundus photography/FFA/OCT

KEY TO MASTER CHART

Sr. No.: Serial number

Sex:

M– Male
F – Female

Treatment group:

1:Case group
2:Control group

Eye affected

RE:Right eye
LE:Left eye
BE:Both eyes
NIL:None of the eyes

Diagnosis at presentation/1m/3m/6m/FFAdiagnosis:

1:Moderate NPDR
2:Severe NPDR
3:Very severe NPDR
4:Early PDR
5:High-Risk PDR

CSME:Clinically significant macular oedema

CMT:Central macular thickness (in microns)

OCT:Optical coherence tomography

BCVA:Best corrected visual acuity

IOP:Intraocular pressure

FFA:Fundus fluorescein angiography

25(OH)Vit D: 25 hydroxy Vitamin D levels (ng/ml)

HbA1c:Glycosylated haemoglobin (in percentage)

FBS:Fasting blood glucose (in mg/dl)

Treatment of DM:

1:Insulin
2:Oral Hypoglycaemic agents
3:Both Insulin and Oral hypoglycaemic agents

Associated systemic diseases:
1:Present
2:Absent

PRP:Panretinal photocoagulation
GRID:Grid laser photocoagulation

1m:1month

3m:3 months

6m:6 months

MASTER CHART

Sr. No.	Name	Age (in years)	Sex	Hospital no.	DURATION OF DM (IN YEARS)	Treatment group	Diagnosis at Presentation - RE	Diagnosis at Presentation - LE	CSME at Presentation	Baseline CMT on OCT - RE	Baseline CMT on OCT - LE	BCVA Baseline - RE	BCVA Baseline - LE	IOP - RE	IOP - LE	FFA diagnosis - RE	FFA diagnosis - LE	FFA diagnosis - CSME	25(OH)VIT D Before Treatment	HbA1c before Treatment	FBS before Treatment	Treatment of DM	Associated Systemic Diseases	Treatment of Retinopathy	Post Treatment 25 (OH) VIT D	HbA1c after treatment	BCVA - RE - 1m	BCVA - LE - 1m	Diagnosis at 1m - RE	Diagnosis at 1m - LE	CSME 1m	BCVA - RE - 3m	BCVA - LE - 3m	Diagnosis at 3m - RE	Diagnosis at 3m - LE	CSME 3m	CMT on OCT 3m - RE	CMT on OCT 3m - LE	BCVA - RE - 6m	BCVA - LE - 6m	Diagnosis at 6m - RE	Diagnosis at 6m - LE	CSME 6m	CMT on OCT 6m - RE	CMT on OCT 6m - LE
1	Ramaswami	60	M	28168	20	1	4	4	BE	339	280	6/36	6/24	16	18	4	4	BE	9.6	8.4	181	1	1	BE GRID PRP	26.61	7.8	6/24	6/18	4	4	BE	6/24	6/18	4	4	BE	329	261	6/60	6/18	5	5	RE	310	185
2	James Isaac	64	M	18406	15	1	1	1	BE	318	243	6/36	6/18	14	14	1	1	BE	25.8	7.3	138	2	2	BE GRID	52.54	6.8	6/36	6/18	1	1	BE	6/18	6/18	1	1	NIL	176	179	6/9	6/18	1	1	NIL	173	179
3	Lakshmi	55	F	17150	15	1	1	2	LE	178	239	6/12	6/18	18	14	1	2	LE	23.2	7.5	156	2	2	LE GRID PRP	50.43	7.1	6/12	6/18	1	2	NIL	6/12	6/18	1	2	NIL	181	177	6/12	6/18	1	2	NIL	176	183
4	Saroja	62	F	24346	10	1	3	3	RE	336	234	6/36	6/12	14	12	3	3	RE	18.7	7.8	153	3	2	BE PRP RE GRID	29.18	7.3	6/24	6/12	3	3	NIL	6/24	6/12	3	3	NIL	176	172	6/24	6/12	3	4	LE	182	239
5	Kasthuri	56	F	35667	20	1	4	4	BE	341	329	6/60	3/60	12	14	4	4	BE	10.7	8.3	171	1	2	BE GRID PRP	26.05	7.9	6/60	3/60	4	4	BE	6/60	3/60	4	4	BE	323	301	3/60	3/60	5	5	BE	334	278
6	Jayalaskshmi	52	F	50179	10	1	4	3	BE	336	317	3/60	6/60	14	14	4	3	BE	17.7	8.2	165	1	2	BE GRID PRP	28.9	7.7	3/60	6/60	4	3	BE	3/60	6/60	4	3	RE	345	178	6/12	3/60	4	4	LE	179	299
7	Maheshwari	54	F	51739	20	1	4	4	BE	264	232	6/24	6/12	14	14	4	4	BE	12.7	8.4	175	1	1	BE GRID PRP	38.19	7.8	6/18	6/12	4	4	BE	6/18	6/12	4	4	RE	256	187	6/18	6/9	4	4	RE	242	176
8	Raju	62	M	52633	5	1	2	2	BE	245	243	6/18	6/18	14	14	2	2	BE	21.8	7.6	147	1	1	BE GRID PRP	46.12	7	6/12	6/18	2	2	LE	6/12	6/12	2	2	NIL	167	175	6/12	6/12	2	2	NIL	181	176
9	Gunasekaran	59	M	36639	15	1	1	1	RE	254	175	6/18	6/6	12	12	1	1	RE	28.2	7.4	141	2	2	RE GRID	56.19	6.7	6/18	6/6	1	1	RE	6/18	6/9	1	1	BE	243	231	6/18	6/9	1	1	LE	179	240
10	Albert	45	M	41340	8	1	2	2	RE	235	181	6/12	6/9	12	12	2	2	RE	21.2	7.5	143	2	2	BE PRP RE GRID	32.13	7.2	6/9	6/9	2	2	NIL	6/9	6/9	2	2	NIL	175	181	6/9	6/9	2	3	NIL	176	182
11	Selvaraj	49	M	46895	10	1	2	2	RE	248	179	6/18	6/9	14	12	2	2	RE	22.3	7.6	149	2	1	BE PRP RE GRID	48.6	7	6/12	6/9	2	2	NIL	6/12	6/9	2	2	NIL	178	186	6/12	6/9	2	2	NIL	184	176
12	Loganathan	53	M	56545	15	1	4	4	BE	276	254	6/24	6/12	18	18	4	4	BE	12.8	8.2	164	1	2	BE GRID PRP	37.9	7.7	6/18	6/12	4	4	BE	6/18	6/12	4	4	LE	179	278	6/18	6/12	4	4	LE	182	243
13	Jyotindra Gandhi	72	M	54549	7	1	2	2	BE	334	324	6/60	6/60	12	14	2	2	BE	22	7.5	139	2	2	BE GRID PRP	48.33	7.1	6/36	6/60	2	2	LE	6/12	6/12	2	2	NIL	184	176	6/12	6/12	2	2	NIL	179	184
14	Munniyammal	55	F	36556	5	1	1	1	RE	327	173	6/36	6/9	14	16	1	1	RE	26.3	6.8	121	2	2	RE GRID	53.21	6.8	6/18	6/12	1	1	NIL	6/18	6/9	1	1	NIL	189	174	6/18	6/9	1	1	NIL	175	182
15	Seetha	65	F	34788	3	1	1	1	NIL	177	181	6/12	6/6	12	14	1	1	NIL	26.6	7.3	134	2	2	NIL	54.33	6.7	6/12	6/6	1	1	NIL	6/12	6/6	1	1	NIL	185	179	6/12	6/6	1	1	NIL	182	175
16	Savitha	66	F	51676	2	1	1	1	NIL	183	176	6/6	6/12	16	14	1	1	NIL	27.5	6.9	123	2	2	NIL	54.21	6.6	6/6	6/12	1	1	NIL	6/6	6/12	1	1	NIL	178	183	6/6	6/12	1	1	NIL	187	179
17	Chandrasekar	70	M	24356	10	1	3	3	BE	236	245	6/12	6/18	14	12	3	3	BE	15.8	7.9	155	1	1	BE GRID PRP	40.92	7.4	6/18	6/18	3	3	RE	6/18	6/18	3	3	RE	245	177	6/18	6/18	3	3	NIL	178	185
18	Kumudha	56	F	54559	15	1	3	3	BE	314	298	6/36	6/12	18	18	3	3	BE	16.2	8	160	1	1	BE GRID PRP	39.54	7.4	6/24	6/12	3	3	NIL	6/24	6/12	3	3	NIL	186	175	6/24	6/12	3	3	NIL	174	182
19	Kalai selvi	67	F	45230	12	1	3	3	RE	247	176	6/18	6/9	14	14	3	3	RE	17.7	7.9	156	1	2	BE GRID PRP	42.14	7.4	6/18	6/12	3	3	LE	6/18	6/12	3	3	NIL	179	183	6/18	6/12	3	3	NIL	185	176
20	Krishnammal	55	F	32667	15	1	3	3	NIL	168	174	6/12	6/18	16	14	3	3	NIL	18.7	7.8	149	1	2	BE PRP	44.34	7.2	6/12	6/18	3	3	NIL	6/12	6/18	3	3	NIL	182	177	6/12	6/24	3	3	LE	179	245
21	Sanaullah	56	M	16170	13	2	4	4	BE	332	326	6/36	6/60	12	12	4	4	BE	9.1	8.4	180	3	1	BE GRID PRP	8.9	8.2	6/36	6/60	4	4	BE	6/36	0.78	5	5	RE	324	182	0.78	6/36	5	5	RE	304	175
22	Muthu	65	M	50274	18	2	4	4	BE	302	327	6/60	6/36	18	20	4	4	BE	10.5	8.4	175	1	2	BE GRID PRP	10.8	8.1	6/60	6/36	4	4	BE	6/60	6/60	5	5	BE	331	314	6/60	6/60	5	5	LE	183	277
23	Sundaram	65	M	49725	22	2	4	4	BE	238	278	6/18	6/24	14	14	4	4	BE	11.1	8.2	161	1	2	BE GRID PRP	11.08	8.2	6/18	6/24	4	4	BE	6/18	6/24	5	5	BE	254	256	6/18	6/18	5	5	BE	243	238
24	Anandhan	48	M	5048	20	2	4	4	BE	341	246	3/60	6/18	18	16	4	4	BE	11.5	8.2	163	1	2	BE GRID PRP	12.81	8.1	3/60	6/18	4	4	BE	6/60	6/18	5	4	NIL	184	174	6/60	6/18	5	4	NIL	179	185
25	Kasinathan	55	M	66304	10	2	2	2	BE	273	268	6/24	6/24	14	14	2	2	BE	18.3	7.5	143	1	1	BE GRID PRP	17.21	7.5	6/12	6/24	2	2	LE	6/12	6/18	2	2	NIL	175	182	6/12	6/18	4	3	RE	246	175
26	Shekar	43	M	64012	7	2	1	1	BE	256	242	6/18	6/12	14	14	1	1	BE	18.8	6.9	123	2	2	BE GRID	17.71	6.7	6/18	6/12	1	1	LE	6/12	6/9	1	1	NIL	173	179	6/12	6/9	1	1	NIL	171	185
27	Ravichandran	48	M	52584	14	2	1	1	BE	328	319	6/36	6/36	18	16	1	1	BE	23.5	6.8	120	2	2	BE GRID	21.42	6.8	6/36	6/36	1	1	BE	6/36	6/36	1	1	BE	278	256	6/24	6/24	1	1	NIL	186	173
28	Kumaran	54	M	49548	15	2	2	2	BE	231	334	6/12	3/60	14	14	2	2	BE	20.5	7.6	162	2	2	BE GRID PRP	22.35	7.4	6/9	6/36	2	2	NIL	6/9	6/12	2	2	NIL	186	176	6/9	6/12	2	5	NIL	174	186
29	Rachel	69	F	15431	12	2	2	2	NIL	187	179	6/18	6/12	18	18	2	2	NIL	22.4	7.5	154	2	1	BE PRP	21.09	7.5	6/18	6/12	2	2	NIL	6/18	6/12	2	2	NIL	189	173	6/18	6/12	2	5	NIL	187	176
30	Mahesh	55	M	60690	5	2	1	1	RE	243	172	6/18	6/6	14	14	1	1	RE	26.5	7.4	143	2	2	RE GRID	24.81	7.2	6/18	6/6	1	1	RE	6/9	6/6	1	1	NIL	176	185	6/9	6/6	1	1	NIL	172	183
31	Kadhiresan	43	M	62087	8	2	1	1	BE	328	316	3/60	6/60	12	12	1	1	BE	29.9	7.3	139	2	2	BE GRID PRP	30.22	7.3	6/24	6/24	1	1	NIL	6/24	6/24	1	1	NIL	178	171	6/12	6/12	1	1	NIL	209	175
32	Rajendran	50	M	60549	10	2	1	1	NIL	174	183	6/6	6/9	14	14	1	1	NIL	42.5	7.4	135	2	2	NIL	41.9	7	6/6	6/9	1	1	NIL	6/6	6/9	1	1	NIL	179	185	6/12	6/9	1	1	RE	245	178
33	Pappathi	55	F	60783	17	2	4	4	BE	236	244	6/12	6/18	14	12	4	4	BE	15.6	8.3	173	1	1	BE GRID PRP	15.31	8.1	6/12	6/18	4	4	BE	6/12	6/12	5	4	NIL	183	179	6/12	6/12	4	4	LE	179	237
34	Jyothi	65	F	61154	5	2	2	2	BE	239	279	6/12	6/24	14	14	2	2	BE	21.3	7.6	139	2	2	BE GRID PRP	23.45	7.5	6/9	6/24	2	2	LE	6/9	6/18	2	2	NIL	185	177	6/9	6/18	2	2	NIL	176	186
35	Devaki	67	F	63445	10	2	3	3	BE	246	256	6/18	6/18	14	16	3	3	BE	15.8	8	153	3	2	BE GRID PRP	16.81	7.8	6/12	6/12	3	3	NIL	6/12	6/12	3	3	NIL	187	175	6/12	6/12	4	4	NIL	175	183
36	Arumugam	54	M	61224	5	2	2	1	NIL	178	183	6/9	6/6	12	12	2	1	NIL	32.3	7.4	138	2	2	RE PRP	30.32	7.4	6/9	6/6	2	1	NIL	6/9	6/6	2	1	NIL	184	173	6/12	6/6	2	1	RE	236	176
37	Nithya	60	F	54089	10	2	3	3	NIL	179	176	6/12	6/9	12	14	3	3	NIL	21.1	7.8	155	1	1	BE PRP	22.31	7.7	6/12	6/9	3	3	NIL	6/12	6/9	3	3	NIL	174	184	6/12	6/9	4	5	NIL	185	179
38	Sakunthala	59	F	56678	7	2	3	3	LE	177	254	6/9	6/18	14	14	3	3	LE	18.4	7.9	149	1	2	BE PRP LE GRID	19.22	7.9	6/9	6/9	3	3	NIL	6/9	6/9	3	3	NIL	172	183	6/9	6/9	4	4	NIL	178	183
39	Shantha	55	F	62455	15	2	3	3	BE	237	242	6/12	6/9	12	12	3	3	BE	17.2	7.9	159	1	2	BE PRP BE GRID	19.19	7.7	6/12	6/9	3	3	BE	6/9	6/9	3	3	LE	182	231	6/9	6/9	4	5	LE	179	245
40	Rajeshwari	64	F	45667	10	2	3	3	BE	316	247	6/36	6/18	16	16	3	3	BE	16	7.8	152	1	2	BE GRID PRP	15.7	7.8	6/36	6/9	3	3	RE	6/18	6/9	3	3	NIL	184	179	6/18	6/9	4	4	NIL	184	172